Pontifícia Universidade Católica
do Rio de Janeiro

Martina Virág Kovács

Effects of Prenatal Alcohol Exposure During the Development of the Central Nervous System: focus on preclinical and clinical studies

Dissertação de Mestrado

Dissertation presented to the Programa de Pós-Graduação em Psicologia of PUC-Rio in partial fulfillment of the requirements for the degree of Master em Psicologia.

Advisor: Prof. Thomas Eichenberg Krahe

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Prof. Thomas Eichenberg Krahe
Advisor
Departamento de Psicologia - PUC-Rio

Profa. Helenice Charchat Fichman
Departamento de Psicologia - PUC-Rio

Prof. Cláudio Carneiro Filgueiras
UERJ

Rio de Janeiro, February 14, 2023.

Martina Virág Kovács

The author graduated in Special Needs Education at University of ELTE (Hungary) in 2012. Since 2019, participates in a research project on Fetal Alcohol Spectrum Disorders at PUC-Rio. The author's main research interest includes neurodevelopment, multisensory integration, and substance use during pregnancy- from the perspective of neuroscience.

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Dedication

For all families suffering from the consequences of prenatal alcohol exposure

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Abstract

Kovács, Martina Virág; Krahe, Thomas Eichenberg (advisor): Effects of Prenatal Alcohol Exposure During the Development of the Central Nervous System: focus on preclinical and clinical studies. Rio de Janeiro, 2023. 108 p. Dissertação de Mestrado - Departamento de Psicologia, Pontifícia Universidade Católica do Rio de Janeiro

Alcohol consumption during pregnancy may damage the development of the fetus, resulting in the most common preventable cause of neurodevelopmental disability in the world: Fetal Alcohol Spectrum Disorders (FASD). The present dissertation aims to discuss the effects of alcohol on the developing CNS through two articles. The first article elucidates the mechanisms and outcomes of the combined alcohol and cannabis exposure in the offspring through preclinical studies. Alcohol teratogenesis is more potent when administered with cannabis and has more negative effects on the fetus than alcohol alone. Recent data demonstrate the interaction of ethanol and cannabis with the Endocannabinoid system, which plays an important role in neurodevelopment and explains the morphological and behavioral changes seen in preclinical studies. The second article is a systematic review that investigates Brazilian research on FASD, focusing on the instruments used for the neuropsychological assessment of individuals with FASD. While developed countries have decades of research on FASD, in Brazil numerous factors slow down the progress of in this and other areas of research. Socioeconomic status, cultural, and geopolitical divergences are some of these factors, which hinder the development, adaptation, and validation of instruments used in the diagnosis and neuropsychological assessment of FASD. In addition, it is worth noting that the socioeconomic vulnerability of the Brazilian population is an important factor in the increase in the occurrence of more severe forms of FASD. The systematic review points to the need to validate neuropsychological tools for the diagnosis and cognitive assessment of individuals with FASD in Brazil, and the participation of a multidisciplinary team in the diagnosis of FASD.

Keywords

Fetal Alcohol Spectrum Disorders (FASD), Prenatal drug exposure, Drug interactions, Endocannabinoid System, Neuropsychological assessment, Brazil

Resumo

Kovács, Martina Virág; Krahe, Thomas Eichenberg: Efeitos da Exposição Pré-natal ao Álcool Durante o Desenvolvimento do Sistema Nervoso Central: foco em estudos pré-clínicos e clínicos. Rio de Janeiro, 2023. 108 p. Dissertação de Mestrado - Departamento de Psicologia, Pontifícia Universidade Católica do Rio de Janeiro.

O consumo de álcool durante gravidez pode alterar o desenvolvimento neural do feto, causando defeitos ao longo da vida. As consequências são diversas e compõe o termo coletivo: Transtorno do Espectro Alcoólico Fetal (TEAF). Esse transtorno é considerado a causa mais comum de deficiência cognitiva evitável no mundo. Estimativas apontam que no Brasil entre 1 e 1,5% das crianças nascem com alterações no sistema nervoso devido à exposição ao álcool *in* útero. O consumo do álcool é frequente entre mulheres grávidas muitas vezes por desconhecimento dos seus efeitos adversos no desenvolvimento do feto. Outra droga comumente utilizada por mulheres grávidas é a maconha com intuito de amenizar o enjoo durante a gestação. A presente dissertação explora os efeitos da exposição pré-natal ao álcool no feto (em conjunto ou não do uso da maconha) em estudos pré-clínicos e clínicos. Dois artigos foram gerados para a realização deste trabalho. O primeiro artigo relata os mecanismos e as consequências do consumo simultâneo de álcool e maconha durante gravidez, cujo efeito é ainda mais nocivo ao desenvolvimento do feto do que apenas a exposição ao álcool. Dados recentes demonstram a interação do etanol e da maconha com o sistema Endocanabinoide, que tem um papel importante no neurodesenvolvimento. Depois do fechamento do tubo neural, que acontece durante a terceira semana da gestação humana, os olhos e o cérebro se desenvolvem do neuroepitélio. Ambos, o álcool e maconha interferem sinergicamente nesse processo via receptores canabinóides, alterando assim a sinalização "sonic-hedgehog", que por sua vez, resulta em alterações morfológicas e comportamentais em modelos animais. Além disso, o artigo relata os mais recentes achados de estudos clínicos sobre a combinação da dose e tipo de constituintes químicos da machonha, bem como os desfechos morfológicos e neurocomportamentais da exposição conjunta do álcool e da maconha. O segundo artigo é uma revisão sistemática que investiga as pesquisas realizadas no Brasil sobre o TEAF, com ênfase nos instrumentos usados para a avaliação neuropsicológica de indivíduos com TEAF. Enquanto países desenvolvidos têm décadas de pesquisa sobre o TEAF, no Brasil, inúmeros fatores comprometem o progresso nesta

e outras áreas de pesquisa. Entre esses fatores podemos citar, divergências socioeconômicas, culturais e geopolíticas, que dificultam o desenvolvimento, adaptação e validação de instrumentos utilizados no diagnóstico e na avaliação neuropsicológica do TEAF. Além disso, vale ressaltar que a vulnerabilidade socioeconômica da população brasileira é um fator importante no aumento da ocorrência de formas mais graves de TEAF. A revisão sistemática aponta para a necessidade da validação das ferramentas neuropsicológicas de diagnóstico e avaliação cognitiva de pessoas com TEAF e da participação de uma equipe multidisciplinar no diagnóstico do TEAF.

Palavras-chave

Transtornos do Espectro Alcoólico Fetal (TEAF), Exposição pré-natal a drogas, Interação de drogas, Sistema Endocanabinoide, Avaliação neuropsicológica, Brasil

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I. Theoretical background

1. Prenatal alcohol exposure

Prenatal alcohol exposure (PAE) may damage the development of the fetal CNS and affect global intellectual ability, verbal functioning, behavioral skills, as well as emotional and self-regulation (Hoyme et al., 2016; May et al., 2020; Wozniak et al., 2019; Ganthous et al., 2017). Since alcohol easily crosses the placenta and blood-brain barrier, it can alter normal embryonic and fetal development. Neuropathological abnormalities associated with prenatal alcohol exposure were observed in neural migration (Delatour et al., 2019), proliferation (Luo & Miller, 1998), oligodendrocyte number, and white matter integrity (Newville et al., 2017). Furthermore, alcohol also interferes with growth factors and synaptogenesis and disrupts neuronal plasticity (Medina, 2011). In addition, alcohol modifies the homeostasis of brain activity levels, as it can activate both excitatory and inhibitory receptors (Delatour *et al.,* 2020).

The teratogenic effects of gestational alcohol effects on the fetus were first described by Lamoine et al. (1968) in France, and soon after by Jones and Smith (1973) in the United States. Both research groups observed specific characteristics of children born to alcoholic mothers (Sulik et al., 1981). The distinct craniofacial alterations (eye malformation, abnormal nasal and upper lip formation, and significantly reduced brain size) were first clearly identified by Sulik et al. (1981) in C57BL/6J mice. The exposure occurred two times with a low dose of alcohol during the embryonal gastrulation stage (corresponding to the third week of human gestation) (Sulik et al.,1981). Since then, an increasing number of studies sought to understand the teratogenic effects of alcohol on the offspring from the basic cellular level to the complex brain functions (for review, see Riley et al., 2005; Wozniak et al., 2019). These studies revealed the heterogenous nature of the outcomes of prenatal alcohol exposure that may be referred to as a continuum named: Fetal Alcohol Spectrum Disorders (May et al., 2020).

Depending on the gestational age at which the exposure occurred, the nutritional characteristics of the mother, the amount and frequency of alcohol

consumption, and the metabolism of the fetus, different damages can occur in the fetal nervous system. These alterations and dysfunctions are known as Fetal Alcohol Spectrum Disorders (FASD), indicating that the manifestations of symptoms are varied and differ among individuals. The most serious consequence is concentrated under the FAS (Fetal Alcohol Syndrome) category, which presents severe cognitive impairment and ocular and facial dysmorphology (short palpebral fissure, epicanthal folds, absent nasal filter, thin upper lip, short/upward nose) (Riley&McGee, 2005). In addition to FAS, other categories vary in severity and the number of symptoms: Partial Fetal Alcohol Syndrome (PFAS), Alcohol-Related Neurodevelopmental Disorder (ARND), Alcohol-Related Birth Defects (ARBD), and Neurobehavioral Disorders of Alcohol Exposure (ND-PAE) (Hoyme et al., 2016; Mattson et al*.,* 2019). The great variety of symptoms can difficult the diagnostic process of FASD and requires future investigations about the neurocognitive and neurobehavioral profile of individuals on the spectrum (Wozniak et al., 2019; Mattson et al., 2019).

International studies of the prevalence of FASD demonstrate differences among countries. Surveys from the United States, Croatia, and Canada report a 1-5% prevalence of FASD. This rate is as high as 23-28% in some developing countries, such as South Africa (May et al., 2020). Another study estimated the global prevalence of FASD at 0.77%, with the highest rate found in Europe and North America, having about 2-5% of children born with FASD in the population (Wozniak et al., 2019). In Brazil, the prevalence of FASD is estimated to be between 1 and 1.5% (Lange et al., 2017). High rate (66,4%) of the Brazilian population between 12 and 65 years old consumed alcoholic beverages during their lifetime and 30,1% consumed alcohol in the past month (Bastos, 2017). Moreover, the recent COVID-19 pandemic may have an invisible collateral effect on FASD spike: increased stress and uncertainty with restricted contraceptive options contribute to unwilled pregnancies with alcohol exposure (Sher, 2020). However, these numbers are considered underestimated due to the difficulty of finding the appropriate diagnostic criteria in the absence of a maternal report of gestational alcohol use. There is a great scarcity in the identification of biomarkers in the field of FASD and the unreliability of maternal surveys make the diagnostic process and estimation of prevalence more difficult (González-Colmenero et al., 2021).

2. Diagnosis and risk factors

The disorder was included recently in the DSM-V system (Diagnostic and Statistical Manual of Mental Disorders) as Neurobehavioral Disorders of Alcohol Exposure (NDAE) (Wozniak *et al.,* 2019). The most commonly used diagnostic system for FASD worldwide, the 4-Digit-Code diagnostic system was developed by the University of Washington in the early 2000s. It measures on a 4-point Likert scale i) growth deficiency (weight and/or height), ii) FAS facial phenotype, iii) CNS dysfunction, and iv) reported alcohol consumption during pregnancy (Astley & Clarren, 2000). With the emergence of new evidence and knowledge accumulated in the 2000s about the disorder, the IOM (Institute of Medicine in the United States) established new subcategories of FASD. In line with these categories, Hoyme (2016) described the updated system for diagnosis. The two systems (4-Digit Code and Criteria according to Hoyme (2016)) show divergences due to differences in the understanding and characterization of symptoms (Astley et al., 2017). The Decision Tree - Decision Tree model was developed in search of an adequate assessment to identify children with alcohol exposure in intrauterine life by Goh *et al.* (2016). The Decision Tree offers practicality in identifying individuals with FASD, even in the absence of facial dysmorphology or the maternal alcohol consumption report. Another positive point is that the recommended assessment instruments are the most used in clinical practice in the United States and can be easily obtained. The variables used in the model are aligned with the criteria for Neurobehavioral Disorders of Alcohol Exposure according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) (Astley et al., 2017).

Although it is not clear why the clinical consequences of PAE vary among individuals, novel findings indicate that genetic predisposition may play a crucial role in the susceptibility to the teratogenic consequences of prenatal alcohol exposure. (Boschen et al., 2021; Mahajan et al., 2016; Fish et al., 2021; Fish et al., 2022). Several studies identified maternal risk factors associated with an increased risk of FASD, such as the family history of alcohol abuse (Ceccanti et al., 2014; Esper&Furtado, 2014), advanced maternal age (Chiodo et al., 2010; Rubio et al., 2008), lower maternal weight and height, and poor nutrition (May et al., 2016) were strongly correlated with the incidence of FASD. Furthermore, lower socioeconomic

status (May et al., 2013; May et al., 2011; Thanh et al., 2013) and concomitant drug use (Skagerstrom et al., 2013; Mallard et al., 2013) also seem to be associated with the disorder.

3. Combined Alcohol and Cannabis Exposure

The recent and ongoing legalization of medical and/or recreational cannabis use in many parts of the world, the increase in marijuana use during the Covid-19 pandemic (Calina et al., 2021; Word Drug Report, 2022), and the high prevalence of misusing both alcohol and cannabis during pregnancy (Page et al., 2022) alert for the upsurge of FASD cases worldwide. The neurocognitive and behavioral deficits associated with prenatal cannabis exposure are demonstrated in several studies (de Salas-Quiroga et al., 2020; Scheyer et al., 2019; Richardson et al., 2016; Wang et al., 2004; Gunn et al., 2016), however, the findings are inconsistent.

The first observations on alcohol and cannabis interaction during the 1970's evidenced cross-tolerance to alcohol among cannabis users. The heavy marijuana user participants became less intoxicated from ethanol than was expected for that dose (Jones&Stones, 1970; MacAvoy&Marks,1975). Since the 1970, a significant increase in simultaneous marijuana and alcohol use was observed in young adults (Terry-McElrath& Patrick, 2018) and the increased cannabis use frequency is associated with more severe alcohol consequences (Wardell et al., 2020). Pregnant women often use cannabis for its anti-nausea effects (Young and Wolff et al., 2018) and have a low-risk perception of the negative cannabis effects on the child (Barbosa-Leiker et al., 2020; McKenzie et al., 2022). Furthermore, half of the pregnant women who report consuming cannabis also report drinking alcohol (Breit et al., 2019).

Studies of cannabis-induced impairments in the developing brain have encountered many limitations, mainly because the human consumption of cannabis can occur in different ways and with different amounts of constituents. The higher potency of cannabis preparations is associated with mental illness, poor academic performance, lung inflammation, and vascular diseases (ElSohly et al., 2016). The Third Brazilian National Survey on Drug Use show that 7,7% of Brazilians between 12 and 65 years old have already used cannabis during their life (Bastos, 2017). Although numbers on combined and simultaneous alcohol and cannabis use during

pregnancy in the Brazilian population are unknown, some studies indicate an increase in drug use among women (Rodrigues et al., 2019; Silva et al., 2021) and high variance in consumption patterns among different regions of the country. Also, legal and illegal drug use was identified as a principal risk factor for fetal death in Santa Casa de Misericórdia in Juiz de Fora/MG (Fideles et al., 2022).

Thus, studies exploring prenatal alcohol and combined drug exposure during pregnancy in developing countries, such as Brazil, are essential to enable valid diagnostic processes and appropriate rehabilitation plans for the affected individuals.

II. Objectives

In line with the theoretical background, the present dissertation will be composed of two parts. The first part aims to discuss the mechanisms and outcomes of combined prenatal alcohol and cannabis exposure on the developing CNS, showing novel findings about the evolvement of the endocannabinoid system in the morphological and neurobehavioral alterations after exposure in preclinical studies. The second part aims to demonstrate clinical studies in the Brazilian population on prenatal alcohol exposure and the neuropsychological assessments used in these studies.

III. Articles section

Article 1

Kovács, M.V., Charchat-Fichman, H., Landeira-Fernandez, J., Medina, A.E., Krahe, T.E. (2023). Combined exposure to alcohol and cannabis during development: mechanisms and outcomes (Accepted Article in "*Alcohol*")

Abstract

Exposure to substances of abuse during pregnancy can have long-lasting effects on offspring. Alcohol is one of the most widely used substances of abuse that leads to the most severe consequences. Recent studies in the United States, Canada, and the United Kingdom showed that between 1% and 7% of all children exhibit signs and symptoms of fetal alcohol spectrum disorder (FASD). Despite preventive campaigns, the rate of children with FASD has not decreased during recent decades. Alcohol consumption often accompanies exposure to such drugs as tobacco, cocaine, opioids, and cannabis. These interactions can be synergistic and exacerbate the deleterious consequences of developmental alcohol exposure. The present review focuses on interactions between alcohol and cannabis exposure and the potential consequences of these interactions.

Keywords: Endocannabinoid system, Prenatal drug exposure, Simultaneous alcohol and cannabis exposure, Drug interactions, Fetal Alcohol Spectrum Disorders (FASD), Cannabinoid receptors

Highlights:

- Cannabis is the most frequently used illicit drug among pregnant women.
- Half of pregnant women who report cannabis use, also report the consumption of alcoholic beverages.
- Early exposure to both alcohol and cannabis is associated to a higher risk of severe developmental outcomes than exposure to either drug alone.
- Alcohol and cannabinoids exert a synergistic effect during early development through the activation of the endocannabinoid system.

ABBREVIATIONS

2-AG: 2-arachidonoylglycerol

ACEA: Synthetic Cannabinoid, CB1 receptor agonist

AEA: anandamide

AM251: Synthetic Cannabinoid, CB1 receptor antagonist

AM630: Synthetic Cannabinoid, CB2 receptor antagonist

Arc: activity-regulated cytoskeleton- associated protein

cAMP: adenosine 3 ', cyclic 5'-monophosphate

Caspase 3: protein that interacts with caspase-8 and caspase-9

CB_1 receptor: cannabinoid receptor 1

CB_2 receptor: cannabinoid receptor 2

CBD: Cannabidiol, a non-psychotropic cannabis constituent

CBP: Nuclear factor, CREB binding protein

CBs: cannabinoids

CDK5: Cyclin-dependent kinase 5

CP 55.940: Synthetic Cannabinoid, CB1 receptor agonist

CREB: cellular transcription factor

DAGL: diacylglycerol lipase

ECBs: endocannabinoids

FAAH: fatty acid amide hydrolase 1

GABA: Gamma-AminoButyric Acid

GPCR: G protein-coupled receptors

GPR55: cannabinoid receptor, activated with the same potency to THC as CB1

HU-210: Synthetic Cannabinoid, CB1 receptor agonist

JNK: JUN N-terminal kinases

JWH133: Synthetic Cannabinoid, CB2 agonist

JZL195: inhibitor of both fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL)

MAGL: monoacylglycerol lipase

MAPK pathway: mitogen-activated protein kinase

NFkB: nuclear factor kappa light chain enhancer of activated B cells

NMDA receptor: ionotropic receptor activated by glutamic acid

pERK1/2: phosphorylated extracellular regulated kinase 1/2

PLA_2: phospholipase A_2

Rac: Ras-related C3 botulinum toxin substrate 1

Rho: a member of the Ras superfamily of small GTPases

Shh pathway: Sonic hedgehog pathway

Smoothened (Smo) receptor: a G-protein coupled receptor, Sonic-hedgehog (Shh) binding

SR141716A: Synthetic Cannabinoid, CB1 receptor antagonist

THC: psychoactive compound of cannabis

WIN 55.212-2: Synthetic Cannabinoid, CB1 receptor agonist

Introduction

Substance use during pregnancy endangers the fetus and may alter neurodevelopment, causing life-long consequences. Cannabis products are the most frequently used illicit drugs among pregnant women, and recent legalization in many states in the United States increased their use during the last decade (Substance Use and Mental Health Services Administration, 2020). Marijuana is commonly used as an anti-nausea remedy during early stages of pregnancy (Dickson et al., 2018). Interestingly, half of pregnant women who report drinking alcohol also report consuming cannabis (Center for Behavioral Health Statistics and Quality, 2015). Despite the high prevalence of simultaneous drug use, little is known about its effects on the maternal and fetal central nervous systems. The cannabis legalization process for medical and recreational use in various countries globally requires a better understanding of the effects of cannabis during pregnancy.

The consequences of alcohol exposure on the developing child are well documented (Riley & McGee, 2005; May et al., 2020; Mattson et al., 2019; Wozniak et al., 2019) and considered the leading cause of avoidable developmental disabilities (Abel & Sokol, 1986). Physical, cognitive, and neurobiological consequences in the fetus following gestational alcohol consumption are collectively known as fetal alcohol spectrum disorder (FASD). The most devastating outcome of FASD is fetal alcohol syndrome (FAS), which is associated with craniofacial and ocular dysmorphology and growth and cognitive deficits. An increasing number of studies demonstrate FAS-like physical alterations after gestational cannabis exposure (Gilbert et al., 2016; Carty et al., 2018; Boa-Amponsem et al., 2019; Fish et al., 2019). This literature suggests common mechanisms and pathways by which both alcohol and cannabis exert teratogenic effects on the developing central nervous system. Both alcohol and cannabis are known as neuroinhibitory drugs. When alcohol is consumed together with other inhibitory drugs (e.g., cannabis, opioids, or γ-hydroxybutyric acid (GHBA)) it synergistically increases its effect (Singh, 2019). However, the mechanisms that underlie the interaction between these two substances and how this interaction leads to specific physical and behavioral features remain to be elucidated. The results of the few studies on this subject indicate the involvement of the endocannabinoid system with an important role of the cannabinoid-1 (CB_1) receptor and sonic

hedgehog pathway. The present review synthesizes recent findings from animal models that were prenatally exposed simultaneously to alcohol and cannabinoids, with a focus on mechanisms and behavioral and physical outcomes.

Epidemiology

Despite preventive campaigns in recent decades, substance use during pregnancy is still relatively frequent. According to the 2019 National Survey on Drug use and Health, 5.4% of pregnant women declared past-month marijuana use and 9.5% declared past-month alcohol use in the United States (Substance Abuse and Mental Health Services Administration, 2020). The survey reported a significant increase in marijuana use but a slight decrease in alcohol use among pregnant women compared with past years. Ko et al. (2015) reported that 16.3% of past-year marijuana users were pregnant and daily users, and more than 10% of pregnant women used marijuana in the past year. Furthermore, the study reported that 70% of pregnant women believe that marijuana use is harmless.

Alcohol-induced physical, cognitive, and structural deficits are commonly known as FASD, but marijuana-induced disabilities or alterations of central nervous system development are not yet characterized or described as a distinct disorder. However, accumulating evidence demonstrates the teratogenicity of Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD), the main constituents of the *Cannabis sativa* plant (Gilbert et al., 2016; Carty et al., 2018; Boa-Amponsem et al., 2019; Fish et al., 2019).

The prevalence of FASD varies among countries and subgroups within populations. Cultural differences may play an important role in this variation. Studies that employed an active case ascertainment method estimated 1-5% of total FASD cases in Canada, Croatia, and the United States among the population. However, this rate reaches 23-28% in South African communities (May et al., 2020). A recent study estimated that the prevalence of FASD among children and youths in the general population is 7.7 per 1000 (Lange et al., 2017). Popova et al. (2019) reported that the prevalence of FASD in subpopulations (i.e., childcare, special care, correctional institutions, Aboriginal, and specialized clinical groups) was 10-40 times higher than in the global general population.

Few data exist in the literature on simultaneous alcohol and cannabis use among pregnant women. However, cannabis is well known to be commonly used as an anti-nausea remedy during pregnancy (Fish et al., 2019). Studies have described higher craving for other drugs among marijuana users (Wang et al., 2019; Hausknecht et al., 2017; Center for Behavioral Health Statistics and Quality, 2015). Interestingly, 46.1% of pregnant women who smoked marijuana in the past year also declared past-month heavy alcohol use. Other studies estimated that 15.3% of women of peak-fertility age (18-29 years) simultaneously used alcohol and marijuana (Subbaraman & Kerr, 2015). Age-specific changes were observed in simultaneous alcohol and marijuana use in past decades, with a significant increase among young adults (Terry-McElrath & Patrick, 2018). The high prevalence of simultaneous use in this age group is particularly concerning because substance use increases unwilling pregnancies (Egan et al., 2019) and consequently may seriously affect the unrecognized fetus.

Neurodevelopmental consequences of alcohol and cannabis prenatal exposure

Alcohol

Alcohol-induced impairments are well documented in the literature with various types of timing and frequency of alcohol exposure (Sulik et al., 1981). Alcohol is highly neurotoxic and easily crosses the placenta and blood-brain barrier, causing changes in gene expression, neuronal proliferation (Luo & Miller, 1998), migration (Delatour et al., 2019), oligodendrocyte number, and white matter integrity (Newville et al., 2017). It also interferes with growth factors and synaptogenesis and disrupts neuronal plasticity (Medina, 2011). Alcohol also alters the homeostasis of brain activity levels by activating both excitatory and inhibitory receptors. Depending on the gestational age at which exposure occurs, nutritional characteristics of the mother, the amount and frequency of alcohol consumption, and the metabolism in the fetus, different types of damage can occur to the fetal nervous system. Also, prenatal alcohol exposure is associated with long-term health and resiliency deficits to adult-onset neurological disease (Bake et al., 2022). Recent studies indicate that genetic background may also play a key role in the susceptibility to the teratogenic consequences of prenatal alcohol exposure. Gene-

dependent differences in alcohol sensitivity were found in the gastrulation-stage of mouse embryos when comparing two C57BL/6 strains. Alcohol-exposed C57BL/6J embryos exhibited a more pronounced transcriptional effect on craniofacial dysmorphology, cell death, and proliferation compared to C57BL/6NHsd ones (Boschen et al., 2021). The latter harbor a spontaneous Dock2 mutation affecting B cell signaling and immune tolerance that is not found in C57BL/6J mice (Mahajan et al., 2016). Similarly, the tumor suppressor protein Tp53 gene deletion protected against alcohol-induced morphological changes in mice and zebrafish embryos (Fish et al., 2021). Furthermore, Bax gene has been identified to facilitate fetal eye and face malformations of mouse embryos following gastrulation-stage exposure to alcohol (Fish et al., 2022). Together these findings highlight the importance of genetic influences and gene–environment interactions associated with the teratogenic effects of alcohol on the developing fetus.

Even brief alcohol exposure during the first trimester of pregnancy can alter normal development of the neural tube and neural crest and lead to specific craniofacial and ocular dysmorphology, growth deficiency, and neurocognitive impairments, which are frequently observed in FAS, the most severe type of FASD (Sulik et al., 1981).

Second-trimester alcohol exposure is associated with alterations of the proliferation and migration of cortical neurons (Delatour et al., 2019; Luo & Miller, 1998) and abnormal oligodendrocyte differentiation (Darbinien et al., 2021). These alterations are thought to lead to neurocognitive and somatosensory impairments among FASD individuals. Furthermore, baboon experiments suggest that alcohol may cause cerebrovascular dysfunction through endocannabinoid signaling, which plays a role in arterial contractility regulation. Vasodilation was observed in midterm baboon fetuses via CB_2 receptor activation after alcohol exposure (Seleverstov et al., 2017; Simakova et al., 2018). However, cerebral vasodilation in baboon fetuses during mid-gestation disappeared by late gestation, suggesting age-dependent crosstalk between alcohol and the endocannabinoid system (Simakova et al., 2018). Recently, Rouzer and Diaz (2022) showed that alcohol exposure during second-trimester of rat gestation induces functional changes in CRF system (the stress peptide corticotropin-releasing factor) activity in a sex-age and

concentration-specific manner and increases the expression of anxiety-like behavior. An in-depth and cohesive description of the endocannabinoid system is provided below.

Studies of third-trimester alcohol exposure reported alterations of synaptogenesis, an increase in apoptosis, and impairments in neuronal plasticity that lead to impairments in learning and memory and anxiety-like behavior (Medina, 2011; Newville et al., 2017; Filgueiras et al., 2010; Baculis et al., 2015).

Cannabis

The effects of alcohol have been widely investigated in recent decades. Studies of cannabis-induced impairments in the developing brain have encountered many limitations, mainly because the human consumption of cannabis can occur in different ways and with different amounts of constituents. The higher potency of cannabis preparations is associated with mental illness, poor academic performance, lung inflammation, and vascular diseases (ElSohly et al., 2016).

The chemical structure of cannabinoids consists of two main groups: phytocannabinoids (which naturally occur in the cannabis plant) and synthetic cannabinoids (human-made substances that are intended for laboratory use or designer drugs with manipulated chemical structures, mimicking psychoactive effects of the cannabis plant (Fish et al., 2019; Musselman & Hampton, 2014). More than 500 different constituents, including 104 cannabinoids, have been identified in the *Cannabis sativa* plant (ElSohly et al., 2016). The most frequently investigated components are THC and CBD. These two main components exert their effects in different ways and have opposing effects. THC acts as a CB_1 receptor agonist, whereas CBD exerts its effects through various pharmacological mechanisms by acting as a CB_1 receptor antagonist, thereby protecting against THC-induced neurodegeneration (Curran et al., 2016). Consequently, the effects of THC and CBD are different. THC exposure appears to cause anxiety-like behavior and impair learning and memory. Studies of CBD found that it can exert protective effects against these THC-induced impairments (Curran et al., 2016). Cannabis products that are available in the illicit market can largely differ in potency. Potency is based on the amount of THC, the main psychoactive constituent of cannabis, that is

necessary to produce a given effect. According to cannabis potency trends (ElSohly et al., 2016), THC concentrations have increased in various preparations in the United States in recent decades while CBD concentrations have decreased.

Cannabis constituents may exert their effects in distinct ways selectively binding to i) G-protein coupled cannabinoid receptors CB1 and CB2 (Felder et al., 1995), ii) G-protein coupled receptor 55 (GPR55) (Lauckner et al., 2008), and iii) transient receptor potential ion and cation channels such as TRPV1, TRPV2, TRPM8, TRPA channels (De Petrocellis et al., 2011). Cannabinoid receptor agonists induce the inhibition of the activity of adenyl cyclase and N-type voltage dependent calcium channels, decreasing the activity of cAMP-dependent protein kinase (Felder et al., 1995). THC acts mainly through CB1 receptors on presynaptic terminals as a partial agonist, resulting in inhibitory synaptic transmission. CBD (cannabidiol) is a negative allosteric modulator of cannabinoid receptors, with a low bounding affinity to CB1R (Kathmann et al., 2006). The increase of CB1R activity by CBD is due to its action on non-CB1 receptor targets that in turn inhibit endocannabinoid inactivation (Alpár et al., 2016). While CBN (cannabinol) is a low-affinity agonist of cannabinoid receptors (Rhee et al., 1997), some studies reported its TRPA1 agonistic and TPRM8 antagonsitic effects (De Petrocellis et al., 2011; Jordt et al., 2004). Synthetic cannabinoids are full CB1 receptor agonists and mimic the effects of THC, with high bounding affinity to CB1 and CB2 receptors. However, synthetic cannabinoids, such as CP55,940, JWH-018, WIN55, 212-2 are more potent than THC (Augstin & Lovinger, 2022).

This increase in potency can have adverse outcomes on the developing child during fetal exposure. A growing number of studies have investigated physical and psychological alterations that are induced by prenatal marijuana exposure (Gunn et al., 2018; de Salas-Quiroga et al., 2020; Raghunathan et al., 2019; Scheyer et al., 2019), suggesting that it impairs endocannabinoid system signaling in the developing central nervous system, representing an early harmful event during development. In the presence of environmental stressors, alterations of endocannabinoid signaling may provoke the emergence of a specific phenotype, but the reason why alterations are not always present after prenatal cannabinoid exposure remains unknown (Richardson et al., 2016). Nonetheless, prenatal

cannabis exposure is associated with lower birth weight, alterations of sleep patterns, problems in executive functions, emotional problems, impulsivity, hyperactivity (El Marroun et al., 2018), poor verbal memory (Ellingson et al., 2021), and impairments in spatial cognition (de Salas-Quiroga et al., 2020).

During development, a heightened expression of CB_1 mRNA was observed in the amygdala and hippocampus of human fetal specimens from mothers with and without history of cannabis use during pregnancy (Wang et al., 2003). These areas are part of the mesocorticolimbic circuit, which plays an important role in emotional regulation, motor function, and cognition. As the mesocorticolimbic dopamine circuits are coupled to CB_1 receptors (Glass & Felder, 1997), Wang and colleagues (2004) sought to know whether prenatal cannabis exposure affects CB_1 and dopamine receptors D_1 and D_2. The study investigated mid-gestation post-mortem brains and found that cannabis exposure significantly reduced D_2 mRNA expression in the amygdala basal nucleus in male, but not female subjects. This reduction was positively correlated with the amount of maternal cannabis consumption. No significant alterations were found in CB_1, D_1, or D_2 mRNA expression in the amygdala and hippocampus. No cannabis-alcohol interactions have been found, however, alcohol contributed to the decreased expression of D_1/D_2 receptors in the striatum (Wang et al., 2004). These results suggest that cannabis may affect brain organization differently in males and females.

Neurobiological studies of first-trimester cannabinoid exposure have found dramatic changes in neurodevelopment. Chick embryos that were exposed to THC during gastrulation exhibited aberrant neural plate formation and alterations of somite, spinal cord, brain, and heart development (Psychoyos et al., 2008). Prenatal THC administration in mouse embryos acted on the development of γ-aminobutyric acid (GABA)ergic interneurons and pyramidal neurons and led to alterations of hippocampal function and deficits in spatial cognition. Impairments in adult mice after prenatal THC exposure showed sexual dimorphism, in which male rats were selectively affected by prenatal THC administration (de Salas-Quiroga et al., 2020). Tortoriello et al. (2014) reported alterations of axon morphology and an imbalance in cytoskeletal dynamics after early THC exposure, leading to miswiring of the brain in human fetuses. The administration of CB_1 receptor agonists in mouse and zebrafish embryos (Gilbert et al., 2016; Fish et al., 2019; Boa-Amponsem et al.,

2019) during the gastrulation period led to anterior neural tube, ocular, and craniofacial abnormalities, similar to manifestations of FASD.

Second-trimester exposure to cannabinoids is associated with a decrease in brain vasculature. Vasocontraction was observed minutes after the mouse embryonic brain was exposed to CP55,940, a frequently used synthetic cannabinoid in preclinical studies (Raghunathan et al., 2019). THC exposure during the third trimester resulted in a smaller inner diameter of the aorta in human fetuses (El Marroun et al., 2010), which may lead to insufficient oxygen and nutrient supply for developing organs. This may explain growth deficiencies that are frequently observed after marijuana exposure in neonates (Calvigioni et al., 2014; El Marroun et al., 2009).

Actions of alcohol and cannabis on the endocannabinoid system in the developing central nervous system

Studies of animal models of FASD have been developed in recent decades to shed light on the mechanisms of alcohol-induced teratogenic effects in the developing central nervous system. These studies have reported epigenetic modifications (for review, see Basavarajappa & Subbanna, 2016), alterations of neuroplasticity (Granato & Daring, 2018; Medina, 2011), and alterations of signaling pathways (Shukrun et al., 2019; Kumar et al., 2010; Zhang et al., 2013). Alcohol exerts effects on the endocannabinoid system (Basavarajappa et al., 1997; Basavarajappa et al., 1998), a fundamental and widespread neuromodulatory system in the brain and peripheral cells. The endocannabinoid system was discovered while studying psychoactive mechanisms of the cannabis plant. This system is activated by both endocannabinoids (endogenously produced ligands) and cannabinoids (constituents of the *Cannabis sativa* plant). Since its discovery, the endocannabinoid system has been widely studied in various contexts, including substance use disorders.

The endocannabinoid system consists of endogenous ligands (e.g., anandamide [AEA] and 2-arachidonylglycerol [2-AG]), cannabinoid receptors (CB_1 and CB_2), interacting proteins, and metabolic enzymes that are responsible for endocannabinoid formation and degradation. Anandamide is synthesized from

phospholipids, such as *N*-acylphosphatidylethanolamine-specific phospholipase D (NAPE-PLD) and degraded by fatty acid amidohydrolase (FAAH). The biosynthesis of 2-AG occurs through diacylglycerol lipase α (DAGLα) and DAGLβ and degraded by monoacylglycerol lipase (MAGL). The endogenous ligands AEA and 2-AG bind to both CB_1 and CB_2 receptors on the presynaptic cell membrane to activate a cascade of negative feedback mechanisms within neurotransmitter systems to maintain homeostasis in the organism. CB_1 receptors are mainly expressed in the central nervous system and are more abundant than CB_2 receptors, which have been detected predominantly in the peripheral nervous system (Basavarajappa et al., 2019; Bukiya, 2019).

Endocannabinoid signaling mechanisms

The endocannabinoid signaling system plays distinct roles and has different mechanisms during fetal development and in the adult brain. The endocannabinoid signaling system plays an important role during neurodevelopment, including neurogenesis, proliferation, migration, axonal pathfinding, and the formation of synaptic connections (Wu et al., 2011; Bukiya, 2019). The endocannabinoid system may exert effects even before conception because it is present in reproductive tissues and affects sperm fertilizing capacity, ovulation, and hormonal production (for review, see Bukiya, 2019).

Rodent and chick studies suggest that cannabinoid receptors are present at early embryonal stages of development. In chick embryos, CB_1 receptor gene expression was detected in the earliest appearing neurons in the hindbrain as early as stage 10 (somitogenesis; a process during embryogenesis, after 33-38 hours of incubation; Hamburger&Hamilton, 1992). At stage 11 (after 40-45 hours of incubation) receptors appeared in the peripheral nervous system and ophthalmic trigeminal placode, followed by the vestibuloacoustic system, epibranchial ganglions, dorsal root ganglia, the ventral forebrain, and the mesoderm (Begbie et al., 2004). In rats, *Cb1r* mRNA expression and CB_1 receptor binding were detected as early as gestational day 11 in some cells of the neural tube and were present in several distinct structures in later stages (gestational day 14-15; Buckley et al., 1998). In humans, evidence shows CB_1 receptor immunoreactivity in the cortical

plate at gestation week 9, whereas CB_2 receptor immunoreactivity was detected only at later stages of the microglia/macrophage lineage (Zurolo et al., 2010). During fetal development, the distribution of CB_1 receptors is atypical and transient compared with the adult central nervous system (Mato et al., 2003; Buckley et al., 1998).

The presence of the endocannabinoid signaling system at early stages of fetal development indicates its involvement in ontogenesis. During fetal development, (endo)cannabinoid-sensing receptors and related enzymatic machinery regulate neural progenitor cell proliferation, neurite outgrowth, and directional guidance (Alpár et al., 2016). In early stages of central nervous system development, both synthesis and degradation activities occur in the same cell, unlike in the adult brain where postsynaptic on-demand ligand release activates cannabinoid receptors that are expressed on the presynaptic membrane. During developmental stages of proliferation and progenitor cell differentiation, the endogenous ligand AEA blocks extracellular signal-regulated kinase (ERK -its activation is required for cell differentiation) through CB_1 receptors, consequently regulating neural cell fate (Rueda et al., 2002). The endogenous ligand 2-AG and the pharmacological activation of cannabinoid receptors increase progenitor cell proliferation and differentiation (Aguado et al., 2005; Aguado et al., 2006; Jin et al., 2004; Galve-Roperh et al., 2013). During axonal growth, 2-AG is abundantly present in growth cones, and MAGL, the metabolic enzyme that is responsible for degrading 2-AG, is at a low-level concentration. MAGL is unable to degrade exogenous cannabinoids, such as THC, that engage cannabinoid receptors and induce abnormal signaling in stabilized axons (Calvignioni et al., 2014).

In the adult central nervous system, the endocannabinoid system acts as a retrograde neuromodulator messenger (Calvigioni et al., 2014). The endogenous cannabinoids AEA and 2-AG are synthesized from the postsynaptic membrane on demand and bind to cannabinoid receptors on presynaptic neuron in a retrograde manner (Basavarajappa et al., 2019). The activation of cannabinoid receptors induces several signaling effects, such as activating mitogen-activated protein kinase (MAPK), inhibiting adenyl cyclase, and altering ion channel activity (Bukiya, 2019).

Simultaneous alcohol and cannabis exposure: neurodevelopmental consequences and mechanisms

The first studies that investigated the combined effects alcohol and cannabis use observed cross-tolerance between the two substances. Heavy marijuana users became less intoxicated from alcohol consumption than non-marijuana users. They also exhibited fewer alcohol-induced neuropsychological deficiencies after co-administration (Jones & Stone, 1970). Participants who simultaneously used both substances exhibited alterations of sleep patterns (Zarcone, 1973), deficits in cognitive and psychomotor function (Manno et al., 1971), and deficits in divided attention tasks (MacAvoy & Marks, 1975). The simultaneous administration of both substances also appears to show amplifying effects (Hansen et al., 2008). Human studies of combined use often report that these individuals use alcohol and marijuana simultaneously for their amplifying effects (Subbaraman & Kerr, 2015; Terry-McElrath & Patrick, 2018). In the developing brain, the precise spatial and temporal coordination of synaptic communication is essential for effective information processing among excitatory pyramidal neurons, inhibitory interneurons, and subcortical afferents (Berghuis et al., 2007). Alterations of these processes may cause long-lasting deficits in the developing brain.

Alcohol and cannabis are both known as neuroinhibitory substances. Alcohol potentiates the GABAA receptor-operated chloride channels by enhancing GABA activity, which results in decreased neural excitability (Allan & Harris, 1987). However, experimental studies indicate that during development, activation of GABAA receptors can be excitatory because of the high intracellular concentration of chloride that produces reversal potentials that are more positive than the resting membrane potential (Ben-Ari, 2002). The combined use of other neuroinhibitory drugs, such as cannabis, synergistically augment the inhibition that is caused by alcohol. Alcohol augments inhibitory neurotransmission through GABA and decreases excitatory neurotransmission through glutamate (Singh, 2019). Furthermore, perinatal alcohol and marijuana exposure acts on inhibitory interneurons in the adult hippocampal formation. Alcohol acts mainly on postsynaptic GABA receptors (e.g., $GABA_A$ receptors become hyperexcitable), and *N*-methyl-D-aspartate (NMDA) receptors become blocked, whereas marijuana mostly targets presynaptic CB_1 receptors. Simultaneous exposure to alcohol and

marijuana dramatically impacts inhibitory processes in the dentate gyrus during adult neurogenesis (for review, see Reid et al., 2020).

Experimental studies of the mechanisms of neurodevelopmental effects of co-exposure to alcohol and marijuana suggest that these two substances share some effects on the same signaling pathways during early brain development, including the sonic hedgehog signaling pathway. This pathway plays an important role in closing the neural tube (i.e., the induction of floor plate differentiation in the anterior midline) and establishing neural identity in the ventral part of the spinal cord and hindbrain (motor neurons and interneurons; Ericson et al., 1996). This signal transduction requires the Sonic hedgehog (Shh) ligand to bind to Patched (Ptch) and to inhibit its inhibition on Smoothened (Smo). Smo is then free to translocate to the primary cilium where associates with Gαi proteins. The inhibition of Gαi on adenyl cyclase (AC) impedes the conversion of adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP) leading to the inhibition of the accumulation of protein kinase A (PKA). This, in turn, helps maintain the Gli activator (Gli A) state, preventing its proteolytic processing into Gli repressor forms (Gli R). Gene transcription needed for normal cell proliferation is promoted by PKA inhibition. Recent studies show that alcohol and cannabis exert its actions through the Shh signaling pathway and may alter normal embryonic development (Fish et al., 2019; Boa-Amponsem et al., 2019; Boa-Amponsem et al., 2020). Both alcohol and cannabis alter this pathway in distinct ways. Alcohol inhibits sonic hedgehog and maintains the Patched repression of Smo (Hong & Krauss, 2012; Burton et al., 2022; Kahn et al., 2017). Cannabinoids have two mechanisms of action: one through the direct inhibition of Smo and one mechanism through CB1 receptor stimulation that leads to the formation of heteromers with Smo (Khaliullina et al., 2015; Amin et al., 2022). This latter mechanism inhibits Smo-Gαi protein signaling and stimulates CB1 receptor-Gαs protein signaling (Fish et al., 2019; Boa-Amponsem et al., 2020). The presumptive model of the mechanism of action after both alcohol and cannabinoid exposure are presented on *Figure A and Figure B*.

First-trimester co-exposure to alcohol and cannabis

Co-exposure to cannabinoids and alcohol is linked to holoprosencephaly

spectrum defects during early developmental stages. Even low doses of alcohol that are administered together with cannabinoids have similar negative outcomes on the central nervous system as high doses of alcohol exposure alone. In zebrafish and mouse embryos, the teratogenic effects of alcohol and cannabinoids converge through the sonic hedgehog signaling pathway and induce ocular and facial dysmorphology (Fish et al., 2019; Gilbert et al., 2016). Another study found that this neurodegeneration was age-dependent in rat pups, with a peak at postnatal day 7 and disappearance by postnatal day 14 (Hansen et al., 2008).

Fish et al. (2019) found that cannabinoids and alcohol during neurulation in zebrafish and mouse embryos collaborate is linked to holoprosencephaly spectrum defects. Cannabinoid exposure occurred through use of the synthetic cannabinoid HU-210 CP 55,940 and phytocannabinoids CBD and THC. The least teratogenic cannabinoid was CBD, which is not a CB_1 receptor agonist, but high-dose exposure caused serious eye defects. During neurulation (gestational day 8 in mice), the eyes and brain develop from the same neuroepithelium. Alcohol and cannabinoid exposure in this developmental period causes craniofacial alterations and abnormalities of brain development *(Figure C)*. Small-eye phenotype was observed after combined CP 55,940 and subthreshold EtOH exposure in zebrafish, an effect observed in mouse embryos. Similar exposure consequences across species (mouse and zebrafish) suggest the same underlying pathogenetic mechanisms of alcohol and cannabinoids. Cannabinoids are dose-dependently teratogenic and enhance alcohol-induced deficits (*Table A*).

Boa-Amponsem et al. (2019) reported the involvement of the sonic hedgehog pathway by administering ethanol (1%) and low dose CB_1 receptor agonist Arachidonoyl-2′-chloroethylamide (ACEA) which induced dysmorphogenesis in zebrafish embryos. Subthreshold ethanol (0.5%) combined with low dose ACEA (3 mg/L) exposure induced microphthalmia and microcephaly in 49% of the exposed animals and significantly increased risk-taking behavior in novel tank diving test. However, in the presence of CB_1 receptor antagonist SR141716A, the FASD phenotype was rescued. In the same study, the administration of subthreshold ethanol (0.5%) and JZL195 (inhibitor of endocannabinoid degradative enzymes) induced FASD phenotype. The rescue of sonic hedgehog mRNA prevented microphthalmia after alcohol and ACEA

treatment. Similar effects were observed in another study that used the same paradigm, showing that alcohol and cannabinoids disrupted the signaling of sonic hedgehog and fibroblast growth factor and altered the development of GABAergic neurons in the forebrain. The simultaneous exposure to a subthreshold ACEA and alcohol modified behavior in zebrafish during the late fry juvenile stage at approximately 2 months of age, and the neurobehavioral alterations were reversible after an injection of *fgf8* mRNA (Boa-Amponsem et al., 2020). Alterations have also been found in the rat hippocampal formation after exposure to a combination of THC and alcohol vapor during gestational days 5-20. In the dorsal CA1 region of the hippocampus, an increase in the number of parvalbumin interneurons was observed, with a decrease in parvalbumin interneurons in the ventral CA1 region. No difference in the ventral dentate gyrus was observed. Parvalbumin interneurons play a role in lateral inhibition, neurogenesis, and network synchrony. Consequently, changes in parvalbumin interneuron density may influence adult neurogenesis, spatial working memory, novel object exploration, and novel object location recognition (Reid et al., 2021).

The administration of lower concentrations of substances may not induce the same effects as discussed above. Breit et al. (2020) used a model of co-exposure to alcohol and THC vapor to mimic e-cigarettes, a popular route of administration among pregnant women. Their study indicated that THC may alter alcohol metabolism, and co-exposure can increase blood alcohol levels. Alcohol also alters THC metabolism. Higher THC-OH metabolite levels were observed after combined drug exposure in rat dams. Interestingly, co-exposure did not affect the gestational length, litter size, sex ratio, or birth weight, although the alcohol-exposed group exhibited a delay in eye-opening. Prenatal THC exposure was associated with lower body weights during adolescence among offspring (Breit et al., 2020).

Second-trimester co-exposure

The development of the fetal brain vasculature system, the neural stem cell self-renewal and differentiation occur during the second-trimester of gestation. Emerging evidence shows that simultaneous alcohol and cannabis (CP-55940, synthetic cannabinoid) exposure may induce premature neural stem cell growth,

alter cell proliferation, and has been associated with acute and delayed decrease in fetal-directed blood flow (Rouzer et al., 2022).

Third-trimester co-exposure

During the third trimester of gestation, the central nervous system undergoes rapid growth (brain growth spurt) by making and breaking synaptic connections, which lasts until late adolescence. Exposure to drugs during this period may interrupt neuronal plasticity and alter the formation and refinement of neural circuits (Medina, 2011). Developmental studies of mouse embryos reported that alcohol increases AEA levels in postsynaptic neurons through transcriptional activation of the NAPE-PLD and glycerophosphodiester phosphodiesterase 1 (GDE1) enzymes. High levels of AEA decrease glutamate release through CB_1 receptors on presynaptic neurons. The decrease in glutamate release results in NMDA receptor hypofunction and cyclin-dependent kinase 5 (CDK5), ERK 1/2, and cyclic adenosine monophosphate response element binding protein (CREB) hyperphosphorylation, which causes the inhibition of Arc and Rac1 expression *(Figure D.)*. These events disturb neuronal circuit refinement and cause lasting deficits in synaptic plasticity (Basavarajappa et al., 2019).

Combined alcohol and cannabis-exposed rats during postnatal days 4-9 (the period of brain growth spurt - human 3rd-trimester equivalent) showed altered motor function in some tasks at later stages of development (Breit et al., 2019). Early motor development was assessed using a grip strength and hindlimb coordination task on postnatal days 12-20 and a parallel bar motor coordination paradigm was applied to examine motor coordination during adolescence (postnatal days 30-32). Cannabinoid effect was observed using CP-55,940 (CP), a CB_1 and CB_2 receptor agonist frequently used in synthetic marijuana preparations as it mimics THC effects. The results of the experiment showed that combined exposure decreased body weight even more than only ethanol-treated rats. Interestingly, only the ethanol-exposed rats showed delayed motor development, while only CP-exposed rats showed advanced motor development. The combined exposure enhanced the ethanol-related motor deficits in some tasks in female subjects, but in other tasks, the initial delay was reduced over time and these rats showed

performance similar to the control group. CP exposure increased BAC (Blood Alcohol Concentration) levels in the co-exposed group, particularly in female subjects, which may also explain the high mortality among co-exposed subjects (Breit et al., 2019). In humans, co-exposure effects on structural connectivity of the developing central nervous system were assessed during adolescence, using the diffusion tensor imaging technique (DTI) (Wade et al., 2020). White matter integrity relies on healthy oligodendrocyte development and CB_1 receptor activation protects progenitors from programmed cell death (apoptosis). Alcohol and cannabis use alters CB_1 activity, disrupting healthy oligodendrocyte development. The results show that co-use of both substances may lead to worse white matter integrity in three tracts, including the inferior longitudinal fasciculus, anterior thalamic radiation, and cingulum cingulate gyrus (Wade et al., 2020). Moreover, Hansen et al. (2008) observed that the co-administration of a mildly intoxicating dose of alcohol (0.5-1.8 g/kg, 1-3.6 g/kg total dose) and THC (1-10 mg/kg) resulted in massive neurodegeneration in rat neonates - an effect similar to that observed after exposure to a high dose of alcohol alone, suggesting an amplification of alcohol's effects by THC. This neurodegeneration was age-dependent, with a maximum level on postnatal day 7 but the absence of this effect on postnatal day 14 (Hansen et al., 2008). The observed neurodegenerative effect was reversible by administration of the CB_1 receptor antagonist SR141716A.

Potential therapeutic implications

Preclinical studies indicate that a CB1 receptor antagonist, SR141716A, may have a protective effect against the effects of combined exposure to alcohol and cannabinoids during development such as behavioral deficits (Boa-Amponsem et al., 2019), morphological phenotypes (Fish et al., 2019), and neurodegeneration (Hansen et al., 2008). Shh mRNA overexpression has a similar effect, as it rescues normal juvenile behavior (Boa-Amponsem et al., 2019), blocks development of microphthalmia phenotype and abnormal midbrain/hindbrain border formation (Fish et al., 2019). A more recent study in zebrafish revealed that fibroblast growth factor fgf8 mRNA overexpression mitigated the behavioral deficits caused by exposure to alcohol and cannabinoids (Boa-Amponsem et al., 2020). These findings may help to elucidate neuronal mechanisms underlying the effects of early exposure

to alcohol and cannabinoids, but could also help to delineate novel therapeutical strategies to prevent long-term developmental deficits.

Human studies concerning the effects of developmental co-exposure later in life are scarce. However, data from the study of Wade and colleagues (2020) show that combined exposure to alcohol and cannabis during adolescence leads to lower white matter integrity across frontolimbic and frontoparietal tracts (Wade et al., 2020). It is suggested that the two substances may disrupt normal myelination - through activation of CB1 receptors - and lead to abnormal white matter formation. Disrupted frontolimbic tracts have been found in individuals suffering from mood disorders (Hermens et al., 2018; Shollenbarger et al., 2015). Developmental alcohol exposure in ferret resulted in increased functional connectivity between the caudal and rostral portions of the posterior parietal cortex - areas that play a role on multisensory integration (MSI) (Tang et al., 2018). More recently, Keum and colleagues (2023) demonstrated that developmental alcohol exposure disrupts MSI in the ferret. Thus, altered white matter integrity should be taken into consideration during neuropsychological evaluation and/or special educational care for individuals with developmental history of co-exposure to alcohol and cannabis. Yet, several treatments for developmental disorders arising from prenatal use of alcohol are available. For instance, methylphenidate, neuroleptics and/or mood stabilizers, stimulants, and choline supplementation to name a few (for a comprehensive review, see Ritfield et al., 2022). However, clinical studies use small sample sizes and show mixed results while describing several side effects. As alcohol disrupts the formation of main neurotransmitter systems, some of the above-mentioned drugs may have the opposite effect compared to the general population. It is important to highlight that no guidelines exist about the optimal psychopharmacological treatments for the FASD population (Ritfield et al., 2022). Perhaps, some of these strategies may be effective in cases of developmental co-exposure. Future studies are needed to shed more light on this issue.

Conclusions

Substance use during pregnancy is associated with alterations in neurodevelopment and long-lasting deficits in offspring. The most used substances

by pregnant women are alcohol and cannabis (Substance Abuse and Mental Health Services Administration, 2020), which are frequently consumed together. Accumulating evidence shows that alcohol and cannabis, when administered together, synergistically alter neurodevelopment from the earliest stages of gestation (Boa-Amponsem et al., 2019; Boa-Amponsem et al., 2020; Breit et al., 2020; Fish et al., 2019; Reid et al., 2021). Recent data suggest the involvement of the sonic hedgehog signaling pathway in interactions between the effects of alcohol and cannabinoids, resulting in growth deficits, craniofacial and ocular dysmorphology (Fish et al., 2019), alterations of hippocampal areas that are associated with learning and memory (Reid et al., 2021), and increases in risk-taking behavior and anxiety-like behavior in animal models (Boa-Amponsem et al., 2019; Boa-Amponsem et al., 2020). Future studies are necessary to understand the effects of simultaneous cannabis and alcohol use in humans by comparing alcohol-only, alcohol+THC, and THC-only groups and the effects of CBD.

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Author Contributions

AEM conceived, designed and supervised the study. MVK collected and analyzed data, and wrote the manuscript. TEK supervised MVK on the preparation and writing of the manuscript, made the figures of the manuscript. JLF assisted with the design of the table of the manuscript. JLF, AEM, HCF and TEK revised and edited the text of the manuscript. All authors revised and approved the final version of the manuscript.

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Competing interest

The authors declare no competing interests.

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Figures and Tables Legends

Figure A

Simplified representation of altered embryonic signaling in the telencephalon after alcohol and cannabinoid exposure. Alcohol inhibits sonic hedgehog and maintains the Patched repression of Smo, inhibiting the shh signaling. Cannabinoids have a direct inhibition of Smo, while stimulating CB_1 receptors that leads to the formation of heteromers with Smo. The latter mechanism reduces Smo-$G_{\alpha i}$ protein signaling and stimulates CB_1 receptor-$G_{\alpha s}$ protein signaling. (Figure adapted from Fish et al. (2019)

Figure B

Local signals in the telencephalon: Fibroblast Growth Factor (Fgf) and Sonic Hedgehog (Shh) (Figure adapted from Petryk et al. (2015); Geng & Oliver (2009))

Figure C

Ocular and craniofacial morphology are altered in normal, alcohol-exposed, cannabinoid-exposed, and co-exposed mouse embryos. Craniofacial features of a) normal, b) alcohol, c) cannabinoids, and d) combined alcohol and cannabinoids exposed mice. b) Alcohol-exposed mice: narrow eyelid openings and forehead, small midface, short nose (nostril deficiency), hair follicles closer to the midline, long upper lip, philtrum deficiency. c) Cannabinoids-exposed mice: minor to moderate coloboma, small jaws, philtrum deficiency. d) Combined alcohol and cannabinoids exposure: severe eye anomalies (anophthalmia), narrow forehead, small jaws, philtrum deficiency, small nose (nostril deficiency). Growth features of drug exposures. e) normal weight and length of control mice. f) Alcohol-exposed mice: reduced weight and length. g) Cannabinoid-exposed mice: reduced weight, no significant change in length. h) Combined alcohol and cannabinoid exposure: reduced body weight and length. (Figure adapted from Fish et al. (2019)).

Figure D

Developmental exposure to alcohol and cannabinoids leads to neurobehavioral defects. a) Alcohol activates gene transcription of GDE1 and NAPE-PLD enzymes 3) Increased AEA levels, c-d) AEA and cannabinoids act through CB1R at pre-synaptic cell membrane, e) Enhanced level of CB1R mRNA expression, f) CB1-mediated Ca2+ channel inhibition, g) Decreased glutamate release, h) NMDAR hypofunction i) CDK/pERK signaling deficit, j) Decreased CREB phosphorylation, and k) Inhibition of Arc, Rac expression. (Figure adapted from Basavarajappa (2019))

Table A.

Morphological and behavioral alterations after combined ethanol and cannabinoid exposure

Figures and Tables

Figure A

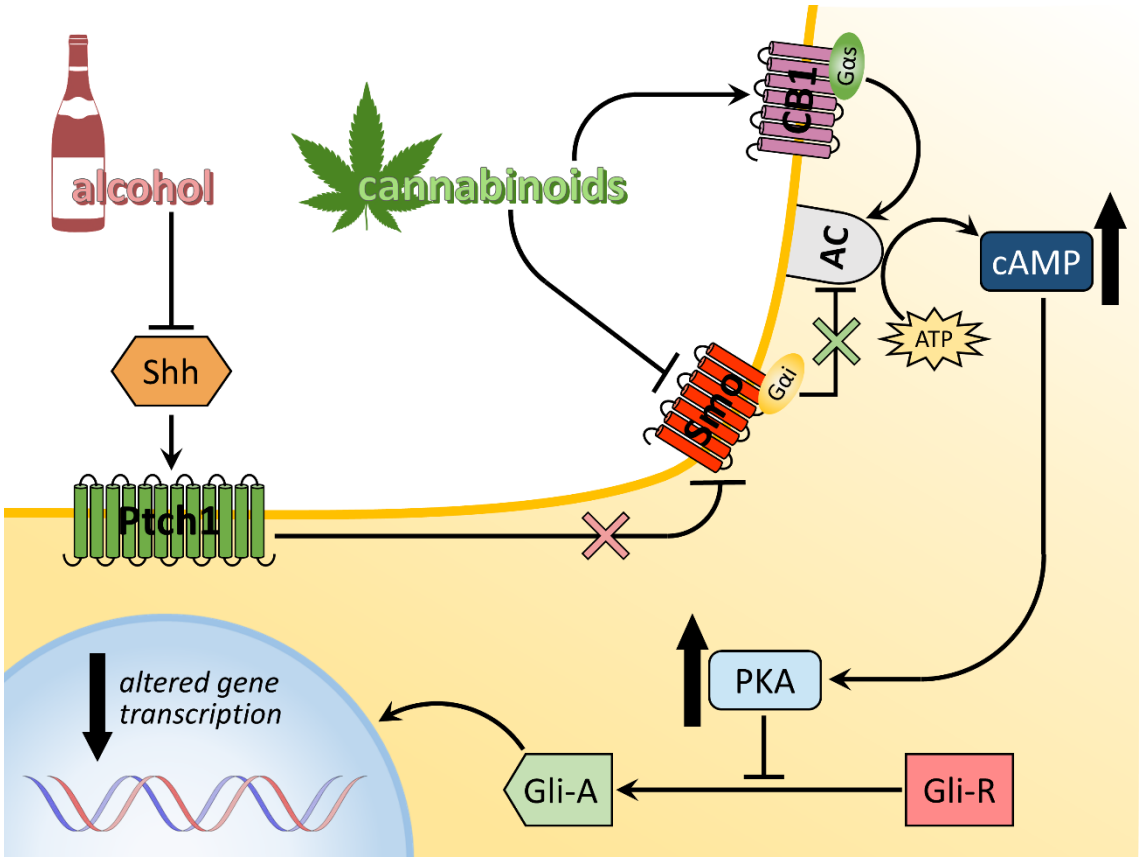

Figure B

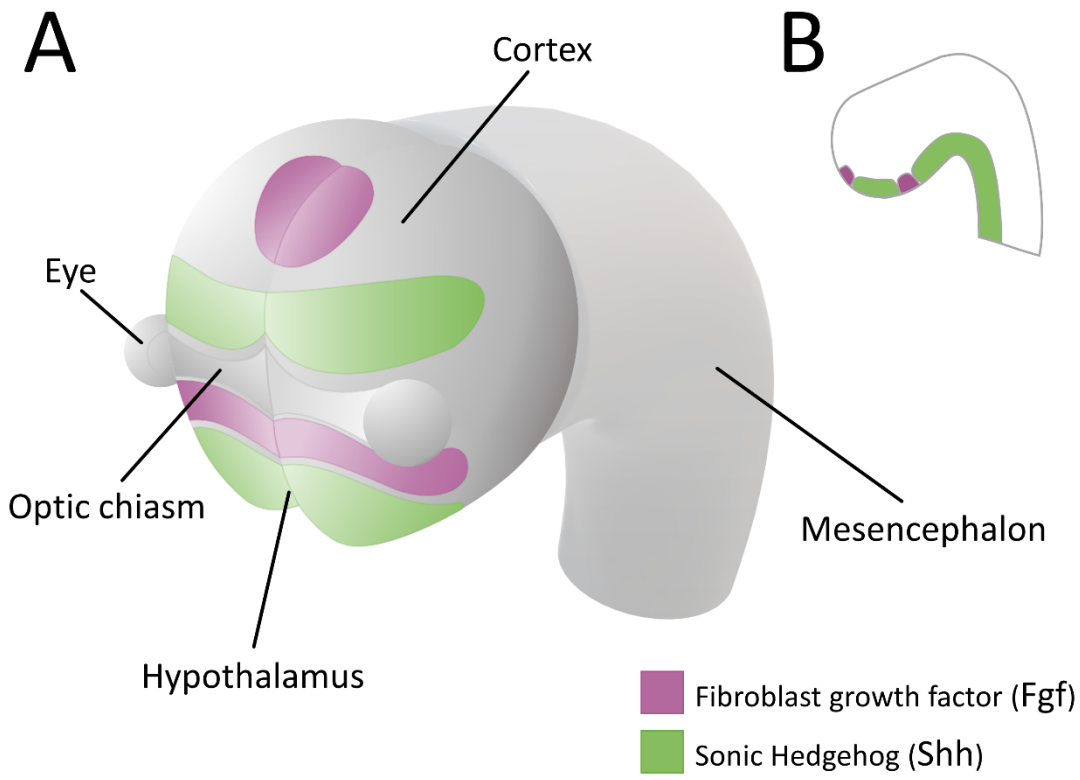

Figure C

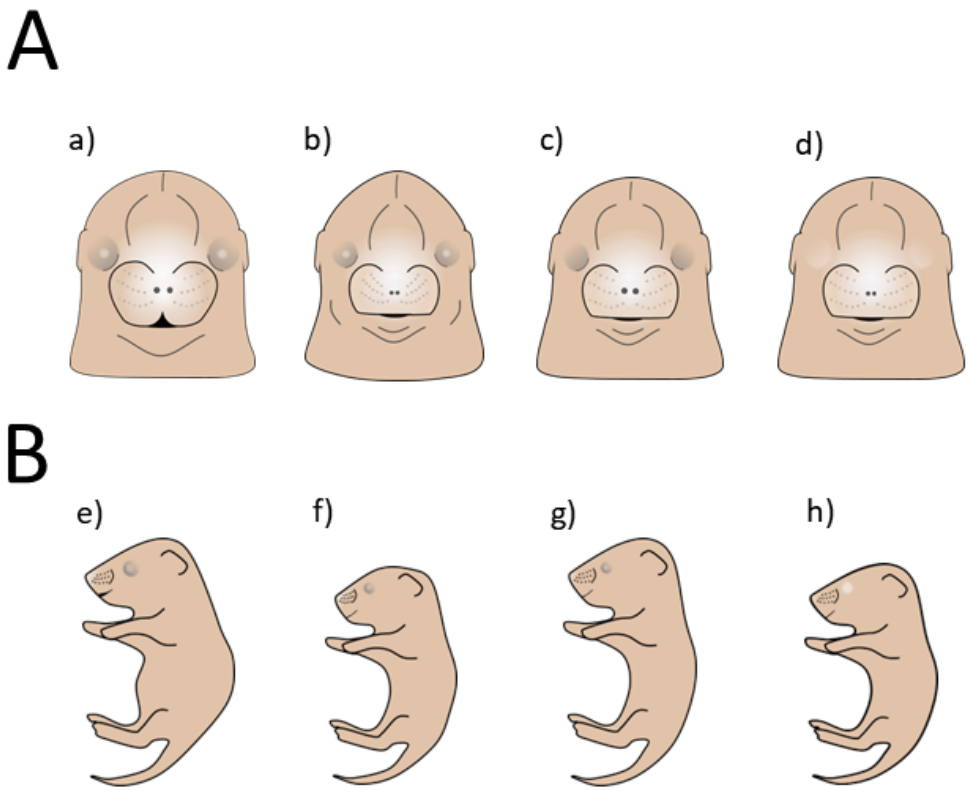

Figure D

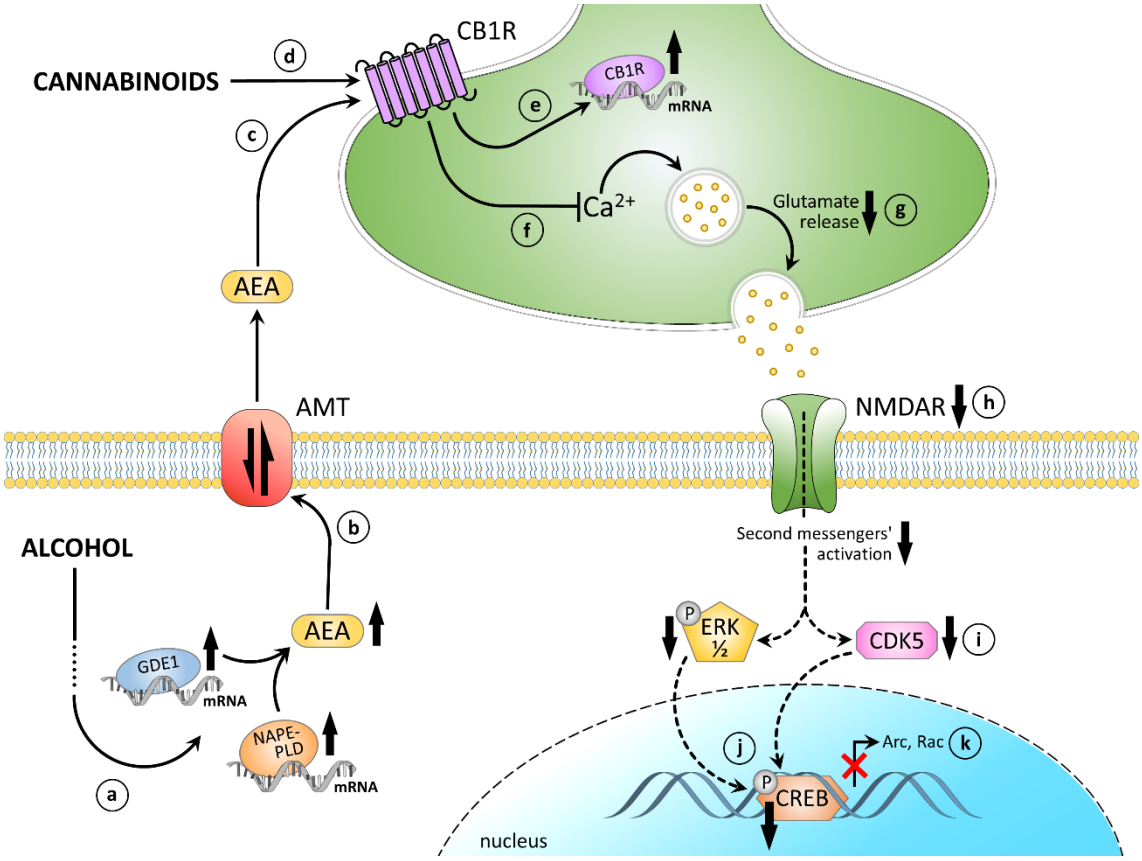

Table A.

Morphological and behavioral alterations after combined ethanol and cannabinoid exposure.

Measure	**Canabinoid (CB) dose**	**Alcohol (Alc) dose**	**System**	**Species**	**Method of analysis**	**Exposure (time)**	**Area /Brain Region**	**Effects**	**References**
Moderate CP-55,940 + Low Alcohol	0.25 mg/kg	1.4 g/kg	In vivo	mouse	dysmorphology assessment	GD 8 (beginning of neurulation)	Eye/Face	Anophthalmia and philtrum deficiency, cleft palate, holoprosencephaly, septal deficiency	Fish et al., 2019
Moderate CP-55,940 + High Alcohol	0.25 mg/kg	2.8 g/kg	In vivo	mouse	dysmorphology assessment	GD 8 (beginning of neurulation)	Eye/Face	Severe eye defects, philtrum deficiency, cleft palate, holoprosencephaly	Fish et al., 2019
Low HU-210 + Low Alcohol	0.03 mg/kg	1.4 g/kg	In vivo	mouse	dysmorphology assessment	GD 8 (beginning of neurulation)	Eye/Face	Near anophthalmia, philtrum and nostril deficiency, cleft palate, brain structural changes, decreased body weight and length	Fish et al., 2019

Low THC + Low Alcohol	0.56 mg/kg	1.4 g/kg	In vivo	mouse	dysmorphology assessment	GD 8 (beginning of neurulation)	Eye/Face	Increased eye defect incidence, decreased body weight and length	Fish et al., 2019
Low CP-55,940 + Low Alcohol	1.0 mg/L	0.5%	In vivo	zebrafish	dysmorphology assessment	5.25-48 hpf (1-2 cell stage)	Eye/Face	No significant craniofacial or ocular alteration was observed after the administration of this dose combination	Fish et al., 2019
Low CP 55,940 + Low Alcohol	2.5 mg/L	0.5%	Ex vivo	zebrafish	Tissue content, real-time quantitative PCR	5.25-48 hpf (1-2 cell stage)	Neural tube	Midbrain/hindbrain defects, small eyes, reduced Shh and Gli1 gene expression	Fish et al., 2019
Moderately high CP 55,940 + Low Alcohol	3.8 mg/L	0.5%	Ex vivo	zebrafish	Tissue content, real-time quantitative PCR	5.25-48 hpf (1-2 cell stage)	Neural tube	Midbrain/hindbrain defects, small eyes, reduced Shh and Gli1 gene expression	Fish et al., 2019
Low ACEA + low Alcohol	3 mg/L	0.5%	In vivo	zebrafish	dysmorphology assessment	6-24 hpf (chronic exposure)	Eye/Face	microphthalmia and microcephaly in 49%of exposed zebrafish	Boa-Amponsem et al., 2019
Low ACEA + high Alcohol	3 mg/L	1%	In vivo	zebrafish	dysmorphology assessment	6-24 hpf (chronic exposure)	Eye/Face	microphthalmia and microcephaly	Boa-Amponsem et al., 2019
Low ACEA + low Alcohol	3 mg/L	0.5%	In vivo	zebrafish	dysmorphology assessment	5.25 – 6.25 hpf (acute adminisatration)	Eye	No significant ocular alteration was observed	Boa-Amponsem et al., 2019

Low ACEA + low Alcohol	3 mg/L	0.5%	In vivo	zebrafish	dysmorphology assessment	8-10 hpf(acute adminisatration)	Eye	microphthalmia	Boa-Amponsem et al., 2019
Low ACEA + low Alcohol	3 mg/L	0.5%	In vivo	zebrafish	dysmorphology assessment	24-27 hpf (acute administration)	Eye	No significant ocular alteration was observed	Boa-Amponsem et al., 2019
Low ACEA + high Alcohol	3 mg/L	1%	In vivo	zebrafish	dysmorphology assessment	5.25–6.25 hpf (first hour of gastrulation)	Eye	microphthalmia	Boa-Amponsem et al., 2019
Low ACEA + high Alcohol	3 mg/L	1%	In vivo	zebrafish	dysmorphology assessment	8–10 hpf (transition gastrulation-neurulation)	Eye	microphthalmia	Boa-Amponsem et al., 2019
Low ACEA + high Alcohol	3 mg/L	1%	In vivo	zebrafish	dysmorphology assessment	24–27 hpf (formation of five-vesicle brain)	Eye	microphthalmia	Boa-Amponsem et al., 2019
Low JZL195 + low Alcohol	2.5 mg/L	0.5%	In vivo	zebrafish	dysmorphology assessment	6-24 hpf (chronic exposure)	Eye	microphthalmia	Boa-Amponsem et al., 2019
Low ACEA + Low Alcohol	1 mg/L	0.5%	In vivo	zebrafish	novel tank diving test	5.25 to 6.25 hpf	behavior	Increased risk-taking behavior	Boa-Amponsem et al., 2019
Low ACEA + Low Alcohol	1 mg/L	0.5%	In vivo	zebrafish	novel tank diving test	8 to 10 hpf	behavior	Increased risk-taking behavior	Boa-Amponsem et al., 2019
Low ACEA + Low Alcohol	1 mg/L	0.5%	In vivo	zebrafish	novel tank diving test	24 to 27 hpf	behavior	Increased risk-taking behavior	Boa-Amponsem et al., 2019
Low ACEA+ Low Alcohol	3 mg/L	0.5%	In vivo	zebrafish	novel tank diving test	5.25 to 6.25 hpf	behavior	Increased anxiety-like behavior, but no alteration in tank diving response	Boa-Amponsem et al., 2019

Low ACEA + High Alcohol	3 mg/L	1 %	In vivo	zebrafish	Eye dysmorphology assessment	8-10 hpf	eye	Small eye phenotype	Boa-Amponsem et al., 2020
Low ACEA + High Alcohol	3 mg/L	1 %	In vivo	zebrafish	Eye dysmorphology assessment	24-27 hpf	eye	Small eye phenotype	Boa-Amponsem et al., 2020
Low ACEA+ Low Alcohol	3 mg/L	0.5%	In vivo	zebrafish	Eye dysmorphology assessment	8-10 hpf	eye	Did not produce microphthalmia	Boa-Amponsem et al., 2020
Low ACEA+ Low Alcohol	3 mg/L	0.5%	In vivo	zebrafish	Eye dysmorphology assessment	24-27 hpf	eye	Did not produce microphthalmia	Boa-Amponsem et al., 2020
Low ACEA + Low Alcohol	1 mg/L	0.5%	In vivo	zebrafish	novel tank diving test	8-10 hpf	behavior	Increased risk-taking behavior	Boa-Amponsem et al., 2020
Low ACEA + Low Alcohol	1 mg/L	0.5%	In vivo	zebrafish	novel tank diving test	24-27 hpf (late neurulation)	behavior	Increased risk-taking behavior	Boa-Amponsem et al., 2020
Third-trimester equivalent									
High CP-55,940 + High Alcohol	0.4 mg/kg/day	5.25 g/kg/day	In vivo	Sprague-Dawley rat	Early motor development task: grip strength trial and Parallel bar motor coordination	PD (postnatal day) 4–9, (chronic administration)	Behavior (motor development and coordination)	Initial motor development delay, severe motor coordination deficit (particularly among females) No effect on grip strength trial	Breit et al., 2019

Abbreviations: PD: post-natal day; BAC: Blood Alcohol Concentration; hpf: hours post fertilization.

Article 2

Kovács, M.V., Lages, Y.V., Charchat-Fichman H., Landeira-Fernandez, J., Krahe, T.E. (2023) Neuropsychological Assessment of Children and Adolescents with Fetal Alcohol Spectrum Disorder (FASD) in the Brazilian population: a Systematic Review

Abstract

Fetal Alcohol Spectrum Disorder (FASD) is a collective name for lifelong physical and neurodevelopmental alterations in the fetus caused by the gestational consumption of alcohol. FASD is considered the most common preventable cause of neurodevelopmental disability in the world. While developed countries have decades of research on FASD, in developing countries - such as Brazil- several factors slow down the progress in this field. Among others, socioeconomic, cultural, and geopolitical divergence greatly difficult the adaptation and validation of psychological evaluation measures. Moreover, the socioeconomic vulnerability -that is markedly present in the Brazilian population- may increase the occurrence of more severe forms of FASD. Therefore, the present review aimed to map and analyze the types of neurodevelopmental assessments used in research on FASD or prenatal alcohol exposure (PAE) in samples of Brazilian children and adolescents. Searches were carried out in the databases Scielo, LILACS, PePSIC, EMBASE, PsycINFO, Web of Science, selecting articles that have investigated the neurodevelopment of children and/or adolescents with FASD in Brazil. The results point to the need for valid neuropsychological assessments while evaluating children and adolescents with FASD in Brazil in order to enable appropriate diagnostic process and rehabilitation paths for this population.

Introduction

In utero alcohol exposure causes long-lasting damage to the CNS. Alcohol is highly neurotoxic and easily crosses the placenta and blood-brain barrier (Wozniak *et al*., 2019). Prenatal exposure to alcohol can cause changes in gene expression, neuronal proliferation and migration, mitochondrial function, interfere with growth factors, synaptogenesis, and disrupt neuronal plasticity (Medina, 2011; Tang *et al.,* 2018; Wozniak *et al*., 2019; Delatour *et al.,* 2020). A large body of the literature support the notion that many factors can influence the magnitude and type of neuronal deficits of early alcohol exposure such as gestational age of exposure, the nutritional characteristics of the mother, the amount and frequency of alcohol consumption, and the metabolism of the fetus, (Mattson et al., 2019; Wozniak et al., 2019). These alterations and dysfunctions are currently known as Fetal Alcohol Spectrum Disorder (FASD), a term that

encompasses all the manifestations of symptoms associated with prenatal alcohol exposure.

A recent study estimated that the global rate of FASD is 0.77% (Wozniak et al., 2018), but there is great variability in the prevalence among different countries. For instance, in the United States, Croatia, and Canada the incidence of FASD is 1-5%. In contrast, the prevalence in South Africa can be as high as 23-28% (May et al., 2020). In Brazil, the prevalence of FASD is estimated to be between 1 and 1.5% (Lange et al., 2019). However, these numbers are likely to be underestimated due to a lack of governmental policies to enforce the use of available diagnostic criteria. Therefore, the prevalence of FASD in Brazil, to a great extent, essentially relies on maternal reports of gestational alcohol use (González-Colmenero et al., 2021). However, looking for ethanol traces in the newborn have been used as biomarkers of prenatal alcohol exposure. Among these, the most frequently used are the biochemical analyses of hair samples (Caprara et al., 2005; Klein et al., 2002), neonatal meconium (Zelner et al., 2013; Chan et al., 2003), placenta (Davis-Anderson et al., 2017; Yoya et al., 2016), and umbilical cord (Través et al., 2007). The accuracy and sensitivity to detect low- moderate levels of alcohol consumption, as well as high level of specificity are the gold standards for prenatal alcohol exposure detection (Bakhireva & Savage, 2011). It is important to note however, that the scarcity of biomarkers in the field of FASD is a worldwide health problem, making the diagnostic process and estimation of prevalence more difficult (Bakhireva & Savage 2011; González-Colmenero et al., 2021). Moreover, another layer of difficulty in the diagnostic process of FASD is the fact that the outcomes of the prenatal alcohol exposure are highly heterogenic (May et al., 2020).

With respect to the diagnostic of FASD the most used system worldwide are the 4-Digit-Code diagnostic system (Astley & Clarren, 2000), and the IOM (Institute of Medicine in the United States) guidelines (Hoyme et al., 2005; see updated guideline: Hoyme et al., 2016). Both diagnostic systems rely on evaluation of the following features: growth deficiency (weight and/or height), FAS facial phenotype, CNS dysfunction, and reported alcohol consumption during pregnancy (Astley et al., 2016). The proper diagnostic evaluation of FASD requires knowledge from multiple areas, including medicine (pediatrics, geneticist, specialist in craniofacial dysmorphology, psychiatrist), psychology (clinical psychology, neuropsychology), speech therapy, special education, and physical therapy, among others (Wozniak et al., 2019; Mattson et al., 2019).

Considering the challenges involving the diagnosis of FASD, Goh and colleagues (2016) have developed a Decision Tree model in search of an adequate assessment to identify children with pre-natal alcohol exposure in the American population. Such model offers accuracy and practicality in identifying individuals with FASD, even in the absence of facial dysmorphology or the maternal report on gestational alcohol consumption. Thus, the recommended assessment instruments of this model are currently the most used diagnostic tool in clinical practice in the United States (Goh et al., 2016). Briefly, the variables used in the model are aligned with the criteria for Neurobehavioral Disorders of Alcohol Exposure according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-V). The study collected data from 434 children (aged 8-16 years) at multiple sites, analyzing over 1000 neuropsychological variables besides facial and ocular dysmorphology features. The following groups were considered: children with alcohol exposure, without alcohol exposure, with/without Fetal Alcohol Syndrome (FAS), with/without other behavioral or cognitive disorders. Validation of the model was performed using another sample of 454 children, divided into two age groups: 5-7 years old and 10-16 years old. The discriminatory validity of the model (i.e., to distinguish children with and without alcohol exposure) was tested with logistic regression (Glas et al., 2003). Post-hoc analyses revealed a high discriminatory accuracy rate of 85%. The diagnostic process of the Decision Tree model relies on two routes, one starting with a pediatrician, and another with a psychologist. Essentially, the physical evaluation is realized by the pediatrician, whereas the psychological evaluation, which is based on IQ tests and neurobehavioral scales can be done by both health professionals. Unfortunately, the Decision Tree model is not currently used in the Brazilian public health system.

In order to gain insight into the FASD research conducted in Brazil, it is necessary to highlight some of the characteristics of the country. Brazil has continental dimensions with an important cultural, geopolitical, and socioeconomic variety that most likely have an important influence on neuropsychological assessments (Guerra et al., 2022). For instance, the socioeconomic factor may play an important role in the prevalence of FASD. Previous findings have demonstrated that low socioeconomic status (SES) is associated with a higher prevalence of severe forms of FASD (May et al., 2022; May et al., 2011; Astley et al., 2000; Thanh et al., 2013). Another relevant issue relies on the fact that the assessment of cognitive functions is strongly culturally dependent. Studies reported that the result of neuropsychological evaluations is influenced by the cultural background of

the participant (Hazin et al., 2016; Fasfous et al., 2013; Er-Rafiqi et al., 2017). Lastly, the fact that the origin of most neuropsychological tests used in Brazil are from developed countries (Guerra et al., 2022), the adaptation and validation of such tests are crucial to guarantee that the outcomes match or at least are similar to those observed at its place of origin (Borsa et al., 2021).

Therefore, this systematic review aims to explore and summarize the existing research on Fetal Alcohol Spectrum Disorder (FASD) conducted in Brazil. Specifically, we ought to gather information on: i) the scientific area of knowledge that has carried out the research on FASD, ii) the use of neurodevelopmental assessment tools for the both the diagnose and/or evaluation of children's abilities with FASD, and iii) whether the applied tools were sensitive and specific enough to detect significant changes in brain functioning associated with prenatal alcohol exposure, and finally iv) evaluate the psychometric properties of the assessment tools used in the selected articles.

Methods

The review proposal has been registered on the Open Science Framework (OSF) webpage (https://osf.io/r2qkt). Studies were identified in Scielo, LILACS, PePSIC, EMBASE, PsycINFO, Web of Science databases that index the scientific literature in the areas of Health and Psychology. The systematic search has been conducted according to PRISMA guidelines (Liberati et al., 2009), and were carried out by two independent researchers (YL and MVK) in March/2022. The descriptors (both in English and Portuguese) have been grouped into three categories: Group A: the population of interest (FASD); Group B: neurodevelopmental assessment; Group C: country of interest (Brazil). Searches were limited to the title, abstract, and keywords. The following search strategy was used to retrieve the articles:

((("alcohol* embryopath*" OR "alcohol* related* neurodevelopmental* disorder*" OR "alcohol* related* birth defect*" OR "fetal* alcohol* effect*" OR "fetal alcohol syndrome*" OR "fetal alcohol spectrum disorder*" OR "foetal* alcohol* effect" OR "foetal* alcohol syndrome*" OR "foetal* alcohol spectrum disorder*" OR "partial fetal* alcohol* syndrome*" OR "partial foetal* alcohol* syndrome*" OR "prenatal* alcohol* expos*" OR "pre-natal* alcohol* expos*" OR "alcohol syndrome, fetal" OR "alcohol syndrome, foetal" OR "fetus alcohol syndrome" OR "Distúrbio* Feta* do Espectro de Alcoolismo" OR "Distúrbio* do Espectro do Alcoolismo Feta*" OR "Transtorno* do Espectro Alcoólico Feta*" OR "Transtorno* Feta* do Espectro de Alcoolismo" OR "Transtorno* do Espectro do Alcoolismo Feta*" OR "Transtorno* Relacionados ao Uso de Álcool" OR "Transtorno* do Sistema Nervoso Induzido* por Álcool" OR "Transtorno* Induzido* por Etanol do Sistema Nervoso" OR "Transtorno* Induzido* por Álcool do Sistema Nervoso" OR "Transtorno* do Sistema Nervoso Induzido* por Etanol" OR "Transtorno* do Sistema Nervoso por Abuso de Álcool" OR "Síndrome Alcoólica Feta*" OR "Síndrome Alcoólica Feta* Parcia*" OR "Transtorno do Neurodesenvolvimento Relacionado ao Álcool" OR "Defeito* Congênito* Relacionado* ao Álcool" OR "Desorde* Neurocomportamenta* da Exposição ao Álcool") AND (“diagnos*” OR “instrument*” OR “assessment*” OR “evaluation*” OR “psych* test*” OR “cognitive dysfunction” OR “motor skill*” OR “psych*assessment*” OR “psych* evaluation*” OR “assessment*, psychological*” OR “neuro-psychological assessment*” OR “neuro-psychological examination*” OR “neuro-psychological test*” OR “neuropsychologic test*” OR “neuropsychological assessment*” OR

"neuropsychological examination" OR "neuropsychological test*" OR "neuropsychology test*" OR "test*, neuropsychological" OR "behavior* observation*" OR "diagnóstico*" OR "teste* psicológico*" OR "avaliaç* psicológica*" OR "Escala* de Avaliaç* Comportamenta*" OR "Teste* Neuropsicológico*" OR "Técnica* de Observaç* do* Comportamento*" OR "Diagnóstico* Diferencia*" OR "Técnica* e Procedimento* Diagnóstico*")) AND (brazil*[Tw] OR brasil*[Tw] OR Acre[Tw] OR Alagoas[Tw] OR Amapá[Tw] OR Amazonas[Tw] OR Bahia[Tw] OR Ceará[Tw] OR "Distrito Federal"[Tw] OR "Espírito Santo"[Tw] OR Goiás[Tw] OR Maranhão[Tw] OR "Mato Grosso"[Tw] OR "Mato Grosso do Sul"[Tw] OR "Minas Gerais"[Tw] OR Pará[Tw] OR Paraíba[Tw] OR Paraná[Tw] OR Pernambuco[Tw] OR Piauí[Tw] OR "Rio de Janeiro"[Tw] OR "Rio Grande do Norte"[Tw] OR "Rio Grande do Sul"[Tw] OR Rondônia[Tw] OR Roraima[Tw] OR "Santa Catarina"[Tw] OR "São Paulo"[Tw] OR Sergipe[Tw] OR Tocantins[Tw]).

Study selection

Titles and abstracts of all studies identified through the database search were independently screened for eligibility by two authors (MVK and YL). Any discrepancies were resolved through consensus or consultation with a third author (TEK). No publication year or language limits were used. Studies were included if the following inclusion criteria were met: (1) it is about the topic of the review, (2) it uses a sample of Brazilian children and/or adolescents with FASD; (3) investigates the neurodevelopment of this population, and (4) is an original clinical study.

Data extraction

The following data were extracted from the selected articles: i) the science area of knowledge that has carried out research on FASD, ii) the neurodevelopmental assessment tools used to diagnose and investigate children's abilities with FASD, iii) whether the applied tools were accurate enough to detect significant changes in brain function due to prenatal alcohol exposure, and iv) evaluation of the criterion validity of the assessment tools used in the study.

Results

A total of 108 citations were identified through the database search after the exclusion of duplicates and a single paper without abstract (Figure 1). Eighty-six additional articles were removed because they were either off topic, did not use the sample of interest, or were not original clinical studies. Sixteen papers were removed because: i) the full text was unavailable, ii) not using a sample of interest, and iii) not making use of neurodevelopmental assessment. Lastly, four articles were added to the list. While they did not appear in any of the six database searches the authors of this review had previous knowledge of the aims, methodological approaches, and results of such studies and thus opted for their inclusion. Altogether, ten articles have been included in the final analysis. The search process is illustrated on the flowchart below (**Figure 1**).

The main characteristics of the articles (authors, year of publication, mean age of the participants- indicating standard deviations, number of the participants, and details on FASD diagnosis) are shown in **Table 1**. Of the ten articles, five were written in English and five in Portuguese. All articles were published in the last 14 years, and studies were conducted mostly in institutions located in the southeast (n = 8) and south (n =1) of the country, with only one from the northeast of Brazil. The age of the samples varied between 3 months and 19 years of a total 648 participants. The wide age gap is due to the inclusion of the article from Momino *et al.,* (2012) in which the sample consisted of adolescents and early adults (13 to 21 years of age). The decision to include the study in the final analysis was because it reveals important data on the cognitive performance (Raven Colored Progressive Matrices Test) of Brazilian institutionalized adolescents. Medical examination as a diagnostic evaluation process was performed in five articles, one of which by a multidisciplinary team. Three articles did not mention the specialist area of the professional(s) who realized the FASD diagnostic process. For instance, Ganthous *et al.,* (2013) reported that a specialist performed the assessment without specifying his/her area of knowledge. Yet, only one article (Nascimento *et al.,* 2007) did not describe details about the diagnostic process.

Regarding the science area of knowledge, half of the selected studies were carried out within the area of speech therapy (Andrade et al., 2013; Garcia et al., 2007; Ganthous et al., 2013; Lamônica et al., 2010; Ganthous et al., 2017), two within the area

of medicine (Ferreria et al., 2013; Momino et al., 2012), one study within the nursing area (Nascimento et al., 2007), and two were performed by a multidisciplinary team (Stromland et al., 2015; Furtado & Roriz, 2016). The multidisciplinary team consisted of neurologists, ophthalmologists, psychologists, psychiatrists, and pediatricians.

With respect to the second objective of this study, we focused on which assessment tools were used to measure the neurodevelopment of individuals exposed to alcohol during gestation. The most frequently used assessment tools and their results are represented in **Table 2**. Altogether, eighteen instruments were identified in ten articles. **Table 3** shows the main characteristics of the instruments and their respective purposes. Four of them were used in more than one article: Rey Complex Figure Test, Wisconsin Card Sorting Test (WCST), Raven Colored Progressive Matrices, and Wechsler Intelligence Scale for Children-Third Edition. Ten assessment tools were developed abroad and then adapted to the Brazilian context and four instruments were constructed in Brazil. However, for the latter we could not find neither their origin nor their validation.

Next, we aimed to describe the results obtained with each assessment tool and investigate whether their use revealed functional alterations in the CNS of children and adolescents that were exposed to alcohol during the prenatal period. The majority of the assessment tools were able to detect impairments in the areas of total IQ scores (Wechsler Intelligence Scale for Children-Third Edition, Raven Colored Progressive Matrices), verbal function (Examination of Language – TIPITI; Routine evaluation protocol of Center for Assistance and Support of Adolescents, Child Language Test - ABFW – Fluency, Observação do Comportamento Comunicativo) visuoperceptual skills (Rey Complex Figure Test), and developmental quotient (Escala de Desenvolvimento Infantil de Heloisa Marinho). It is important to note that some of the instruments showed a floor effect: Peabody Picture Vocabulary Test, Gesell and Amatruda Behavioral Development Scale (Lamônica et al., 2010). In the case of the Child's Phonological assessment (AFC in Portuguese) and Illinois Test of Psycholinguistic Skills, the participants with more severe forms of FASD could not understand the instructions (Garcia et al., 2007). Moreover, some of the assessment tools did not show significant alterations between FASD/PAE group and the control group: Raven's Progressive Matrizes (Ferreira et al., 2013), Wechsler Intelligence Scale for Children-Third Edition, Wisconsin Card Sorting Test (WCST), Rey Auditory Verbal

Learning Test (RAVLT) (Furtado & Roriz, 2016).

Finally, we investigated the criterion validity of the tools and adaptation studies to the Brazilian conditions. There are two types of criterion validity: i) concurrent validity which indicates to what degree an assessment tool correlates with another – i.e., compared to measures that were already validated, and ii) predictive validity that measures the capacity of the tool to predict future performances (AREA, APA, NCME, 2014). Confirmed criterion validity was found for only two instruments: Peabody Picture Vocabulary Test and the Wechsler Intelligence Scale for Children-Third Edition.

To illustrate the quality of the Brazilian versions of the assessment tools, we also investigated other validity and reliability evidence (**Table 3)**. Of the eighteen assessment tools, 10 have a Brazilian adaptation and some evidence of reliability and validity. Included in this category are: Raven Colored Progressive Matrices, Raven Progressive Matrices, Wisconsin Card Sorting Test (WCST), Rey Auditory Verbal Learning Test (RAVLT), Wechsler Intelligence Scale for Children-Third Edition, Rey Complex Figure Test, Peabody Picture Vocabulary Test, Illinois Test of Psycholinguistic Skills, d2 Test of Attention, Test of Semantic and phonetic Verbal Fluency (animals). The description of the adaptation and validation studies for each assessment tools are shown in **Table 3**.

Finally, six of the assessment tools investigated in the present study were valid on the SATEPSI list of the current year: Raven's Progressive Matrices, Wisconsin Card Sorting Test (WCST), Rey Complex Figure Test, Raven Colored Progressive Matrices, d2 Test of Attention, Rey Auditory Verbal Learning Test (RAVLT). Table 3 shows details about this topic. For this particular analysis, information on the technical and scientific quality of psychological assessments was gathered from SATESPI (Psychological Test Evaluation System) official page which is based on the Federal Council of Psychology and lists favorable and unfavorable assessment tools (https://satepsi.cfp.org.br/Lista_Teste_Completa.cfm). Even though we are aware that not all neurodevelopmental evaluations are realized in the field psychology, we consider the SATEPSI list as an important indicator of quality of assessments performed in Brazil. Besides, the SATEPSI offers information about the current status of assessment tools i.e., validation and standardization studies - thus providing an important source of information for future studies and clinical evaluations.

Discussion

The present work aimed to describe the availability and reliability of clinical studies on Fetal Alcohol Spectrum Disorder (FASD) in Brazil. Aside from the scarce number of studies that fulfilled our selection criteria, an observation that stands out from the selected sample is the lack of reliable information about the specific area of practice of the persons performing FASD diagnoses. International recommendations highlight the necessity of multidisciplinary teamwork while establishing the diagnosis (Hoyme et al., 2005, Hoyme et al., 2016, Goh et al., 2016). However, this does not seem to be the case for studies carried in Brazil, an issue of concern. In the present review, besides two studies that followed international recommendations and made use of a multidisciplinary team, (Stromland et al., 2015; Furtado & Roriz, 2016). The science area of knowledge of the research was often inferred by the affiliation and specialist area of the authors, but sometimes from information included in the main text of the manuscript.

Since FASD represents a spectrum of disorders with varying psychopathologies, clinical manifestations, and associated comorbid conditions, proper assessment of neuropsychological functions is essential to determine the correct diagnostic and treatment pathway (for details on diagnostic subcategories of FASD, please see Mattson et al., 2019). In this regard, a variety of neuropsychological assessment tools have long been used to obtain normatively informed and performance-based understanding of various cognitive skills such as attention, memory, and language of individuals suffering from different cognitive disorders, including individuals with FASD (Mikadze et al., 2019; Harvey, 2012; Donders, 2020; Georgiou et al., 2022). While two of the selected studies (Ferreira et al., 2013; Furtado & Roriz, 2016) used the Weschler Intelligence Scale (WISC-III) for IQ evaluation, one of the recommended assessment tools included in the Decision Tree model (Goh et al., 2016), the majority of selected studies do not provide much information about diagnostic processes – including the description of assessment tools. Moreover, none of the neurobehavioral scales (CBCL or VABS, Goh et al., 2016) were applied in the selected studies. Yet, it is important to recognize that this was not the aim of the studies. Instead, some articles sought to collect diagnostic data of PAE or were investigating neurodevelopmental particularities of FASD children and adolescents (**Table 3)**.

The review also sought to describe whether the instruments and protocols used to assess alterations in brain function and behavior. In general, for most neuropsychological

assessment tools, results showed that FASD individuals have a significantly poorer performance compared to controls or to what is expected for a particular developmental period. In spite of that, an issue that deserves attention concerns the floor effect seen in the study of Lamônica and colleagues (2010) that used the Peabody Picture Vocabulary Test and the Gesell and Amatruda Behavioral Development Scale. This was probably due to the small sample size and differences in severity of symptoms. The study sample consisted of five brothers, two of them diagnosed with FAS, the most severe form of FASD (Riley & McGee, 2005; Mattson et al., 2019). Yet, it was also reported that the other 3 brothers had difficulties to understand and follow the instructions of the assessment tools. In contrast, using a larger sample size, Nardelli et al (2011) did not observed similar floor effect and demonstrated that FASD individuals have lower scores on the Peabody Picture Vocabulary Test compared to controls. Another unexpected result from the selected studies that deserves consideration was the lack of significant differences in test performances between PAE and non-PAE groups reported by Furtado & Roriz (2016).A possible reason for this finding may rely on the exclusion of children with IQ < 70. A number of studies have demonstrated that individuals with FASD display poor performance in Assessment of Verbal learning tests (Connor et al., 2000; Crocker et al., 2011; Gross et al., 2018). Perhaps the only exception from Furtado & Roriz's work (2016), was the observation of significant group differences in the Rey Complex Figure Test, in agreement with previous results from Stromland et al. (2015).

While the differences in the outcomes of the same or similar neuropsychological assessment tools may be due to the small sample size of the studies included in this review, one may question the validity and reliability of the instruments used to evaluate differences in brain function and behavior. In this regard, several studies point out that the evaluation of cognitive abilities is significantly influenced by cultural factors (Hazin et al., 2016; Fasfous et al., 2013; Er-Rafiqi et al., 2017). Indeed, Brazilian studies demonstrate that children's performance vary by region, rural vs. urban context (Hazin et al., 2016; Santos et al., 2005). Others showed that family socioeconomic status impact executive functions of children (Burges et al., 2013; Sallum et al., 2017; Sarsour et al., 2011; Schulte et al., 2022). Taking into consideration that most of the neuropsychological tests used in the selected studies were built and designed for populations in developed countries, adaptation and validation to Brazilian conditions is of fundamental importance. Some of the selected articles made reference to the studies responsible for the adaptation

and validation of the neuropsychological tests used in the diagnosis and evaluation of children and adolescents with FASD. Of note, the majority of adaptations has been done in the south regions of the country, which in turn could compromise their efficacy in other parts of Brazil, such as the central and northeast regions. Future studies are warranted to characterize the efficacy and clinical effectiveness of these tests in diverse population across the Brazilian territory.

Conclusion and future directions

In conclusion, this systematic review aimed to evaluate the neuropsychological tools available to diagnose and study the development of Brazilian children and adolescents with FASD. Our findings indicate that a limited number of studies and neuropsychological tools, including standardized tests and functional assessments, are available to diagnose and study of FASD in Brazil. Thus, the available evidence suggests that there is a need for further research to determine and expand the use of appropriate tools for diagnosing FASD, particularly in terms of sensitivity and specificity. The results also highlight the importance of using a multidisciplinary team in the diagnosis of FASD, incorporating a range of different assessment tools and perspectives, in order to accurately identify and understand the developmental impacts of FASD in Brazil. However, some caution is needed in interpreting our data as the scarce literature on the topic does not necessarily imply in low use of neuropsychological tests in the diagnosis/evaluation of cognitive disabilities, including FASD. The neuropsychological tools cited in this review and many others are regularly used in private and public health institutions across the country. Nevertheless, studies on the standardization and effectiveness of neuropsychological tests for early detection and treatment of FASD are of extreme importance. Early detection allows for timely interventions to mitigate the effects of FASD and improve the chances of a positive outcome. Standardization of tests leads to increased reliability and validity, providing more accurate diagnoses, which can lead to more tailored and effective treatments. Access to treatment for FASD and other mental health disorders is of utmost importance for all individuals of the Brazilian population.

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Tables and Figure Legends

Figure 1

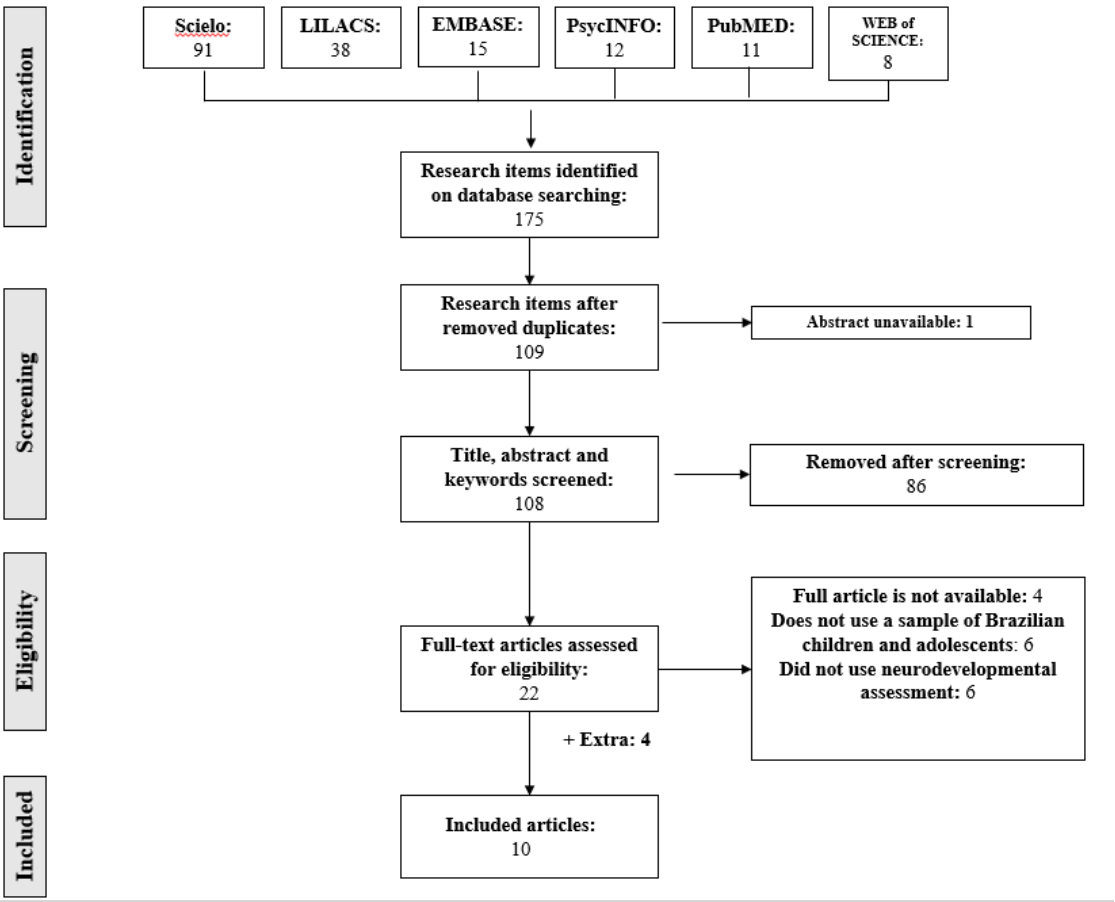

Table 1

First author	Year of publication	Region	Age *M* (*SD*)	N	FASD diagnosis
Nascimento	2007	Rio de Janeiro	14,9 (2,09)	6	Does not describe diagnostic details
Garcia	2007	São Paulo	12 (5,65)	2	Geneticists: positive maternal alcohol use report and evaluation of clinical signs
Ganthous	2013	Marília (SP)	8 (2,74)	18	Specialist evaluated: growth deficiency, facial phenotype, microcephaly, and intellectual disability
Andrade	2013	São Paulo	17	1	Diagnosed in early childhood, using the guideline of the National Research Council, considering growth retardation, neurodevelopment impairment, and facial dimorphism (at least two characteristics must be present such as microphthalmia or short palpebral fissures, maxillary hypoplasia, microcephaly or thin upper lip), intelligence and behavior impairment.
Ferreira	2013	Rio de Janeiro	9,7 (3,02)	10	Growth deficiency, facial characteristics, dysfunction of the CNS, maternal alcohol consumption report. Evaluated by physician.
Lamônica	2010	São Paulo	4,9 (2,25)	5	Physician, through observation of clinical signs and positive report on gestational alcohol use
Momino	2012	Porto Alegre	from 13 to 21 years of age	416	Physician evaluated clinical signs according to IOM criteria (Hoyme et al., 2005) and positive report on gestational alcohol use
Stromland	2015	Recife	6,2	94	Multidisciplinary team evaluated the subjects using the guidelines of the Institute of Medicine (IOM) (Hoyme et al., 2005), height, weight, occipitofrontal circumference measured by anthropometric digital scale, neurological, facial signs assessment
Furtado	2016	Ribeirão Preto	11,9 (0,36)	56 (n=28 prenatal alcohol exposure and n=28 non-exposed	Prenatal Alcohol exposure was confirmed using the maternal alcohol use report (T-ACE) and ICD diagnosis for alcohol harmful use or dependence.
Ganthous	2017	Marília (SP)	10,7 (3,33)	40	4-digit code made by a physician, does not describe the assessment of the functioning of the CNS

Table 2

Name of the assessment tool	Identified alterations in the FASD/PAE group?	References
Wechsler Intelligence Scale for Children-Third Edition (Escala de Inteligência Wechsler para Criança (WISC-III))	Mean Total IQ of the FAS group is on the limit or below typical development. Main deficits were observed on the sub areas: Freedom from Distractibility Index, Perceptual Organization Index, Verbal Comprehension Index, Processing Speed Index[1]. No statistically significant differences between groups, but slightly lower verbal IQ in the PAE group [2]	[1]Ferreira et al., 2013. [2]Furtado & Roriz, 2016.
Wisconsin Card Sorting Test (WCST)	Cognitive functions below average[3]. No statistically significant differences between groups, but slightly lower verbal IQ in the PAE group[2].	[3]Stromland et al., 2015. [2]Furtado & Roriz, 2016.
Rey Complex Figure Test	PAE groups had significantly lower performance on copying the figure (constructive praxis), and memory recovery after 3 min (immediate visual recall) compared to non-PAE group. No differences were found in planning task between groups[2]. Performance was below average. The study does not describe details about the participants' performance[3].	[3]Stromland et al., 2015. [2]Furtado & Roriz, 2016.
Raven Colored Progressive Matrices (Special Scale) (Angelini et al., 1999)	Below average. The study does not describe details about the participants' performance[3]. Lower IQ scores of institutionalized group with European ancestry[4].	[3]Stromland et al., 2015. [4]Momino et al., 2012.

Table 3

Instrument	Objetives of the instrument	Brazilian Standardization	Validity and Reliability	Found alterations in PAE/FASD group?	References
Heloisa Marinho's Child Development Scale	Assesses the development of children aged 0 to 8 years and 11 months in three areas: physical, mental, and social areas	Developed in Brazil by Heloisa Marinho (1997)	Not found	Developmental Quotient of FAS children and adolescents is below the expected by their age	Nascimento *et al.,* 2007
Child Language Test - ABFW - Fluency	Evaluates common disfluencies and stuttering disfluencies in the oral narrative of children between 2-12 years of age. The test consists of 18 assessment blocks.	Developed in Brazil by Andrade et al., 2000	Not found	FASD group showed more dysfluency: hesitation and longer pauses during narration	Ganthous *et al.,* 2013
Observation of Communicative Behavior (OCB)	Evaluates the interaction with the evaluator in a playful situation. Qualitative observation of communicative functions in speech therapy assessment.	Not found	Not found	Difficulties in communicative behavior were pointed out in the qualitative description of FASD children	Lamônica *et al.,* 2010
Peabody Picture Vocabulary Test	Evaluates the lexical development in the receptive-auditory domain of children aged 2 and a half to 18 years old, using non-verbal responses.	Capovilla et al., 1997. (The complete form of the Brazilian version of the Test is not available due to copyright issues. The frequently used version is called "Teste de Vocabulário USP")	Convergent validity: it significantly correlated with "Teste de Competência de Leitura de Palavras e Pseudopalavras" (TCLPP1) (Capovilla & Prudêncio, 2006)	The test had a floor effect, only one participant was able to reach scores below average	Lamônica *et al.,* 2010
Gesell and Amatruda Behavioral Development Scale	Assesses the development of children between 4 weeks and 36 months in five areas: adaptive behavior; gross and fine motor; language; personal-social behavior	Not found	Not found	The application of the scale was not possible in one of the five participants. The lowest scores were obtained in the domains of adaptive behavior, social behavior and language behavior.	Lamônica *et al.,* 2010
Raven's Progressive Matrices	It evaluates general non-verbal intelligence of individuals from 5	Raven, 2002 (Manual)	Internal validity: Cronbach's alpha	Most of the students perform within the normal range.	Ferreira et al., 2013

	years of age to eldery. The instrument comprises 5 series (A, B, C, D, E) with 60 multiple choice questions with increasing difficulty.		=0,91 (Flores-Mendoza et al., 2014). Exploratory factor analysis showed eight factors with eigenvalues greater than 1. (Flores-Mendoza et al., 2014)		
Oral narrative	Assesses the micro- and macrostructural aspects of speech and the level of global coherence of the story.	Not found	Not found	Worse performance of FASD groups on the aspects of micro - and macrostructure was observed.	Ganthaous *et al.,* 2017
Examination of Language - TIPITI (Braz & Pellicciotti, 1988)	Speech-language evaluation; areas: Word Categorization, Words Definition, Immediate auditory memory, Completion of sentences, Reading words, Reading text. Assess from 3 to 18 years of age.	Developed in Brazil by Braz& Pellicciotti (1988)	Not found	The participant had significant alterations on the receptive and expressive fields of both oral and written language.	Andrade et al., 2013
Routine evaluation protocol of Center for Assistance and Support of Adolescents	Language evaluation; areas: dictation, free diction, oral understanding, written understanding, phonological awareness.	Not found	Not found	The participant could not realize the phonemic exclusion and phonemic transposition tests, but did correctly the syllabic synthesis, phonemic synthesis and rhyming tasks	Andrade et al., 2013
Wechsler Intelligence Scale for Children-Third Edition (Escala de Inteligência Wechsler para Criança (WISC-III))	Evaluate cognitive abilities of children and adolescents between ages 6-16, consist of 3 composite IQ scores: Full Scale IQ, Verbal IQ, and Performance IQ; and four index scores: Freedom from Distractibility Index, Perceptual Organization Index, Verbal Comprehension Index, Processing Speed Index; and 13 subtests: 13 subtests	Figueiredo, Pinheiro& Nascimento, 1998	Confirmed Predictive validity (school grades) and Concurrent Validity: with Raven Colored Progressive Matrices)(Cruz, 2005). High reliability for cubes CCI= 1,00; Picture	Mean Total IQ of the FAS group is on the limit or below typical development. Main deficits were observed on the sub areas: Freedom from Distractibility Index, Perceptual Organization Index, Verbal Comprehension Index,	Ferreira et al., 2013 Furtado &Roriz 2016

	(Information, Similarities, Arithmetic, Vocabulary, Comprehension, Digit Span, Picture Completion, Coding, Picture Arrangement, Block Design, Object Assembly, Symbol Search, and Mazes		arrangments CCI=0,99; Digits CCI=0,97; Object assembley CCI= 0,91; Picture Completion CCI=0,88; Information CCI=0,88; Arothmetic CCI=0,79; Comprehension CCI=0,76; Similarities CCI=0,74; Vocabulary CCI=0,71 (Araújo&Figueiredo, 2013). Internal validity: Cronbach's alpha = 0,94 (Figueiredo, Pinheiro& Nascimento, 1998)	Processing Speed Index (Ferrera et al., 2013). No statistically significant differences between groups, but slightly lower verbal IQ in the PAE group (Furtado & Roriz, 2016).	
Child's Phonological assessment (Avaliação Fonológica da Criança (AFC))	Evaluates phonological acquisition of children from 3 years of age. It consists of five thematic figure groups: vehicles, bathroom, kitchen, living room, zoo. The figures may provoke the spontaneous naming of 125 distinct words.	Brazilian test developed by Yavas et al.,1992	Not found	The absence of oral speech of one participant made the assessment impossible. The second participant showed limited capacity in understanding the orders.	Garcia et al., 2007
Illinois Test of Psycholinguistic Skills (Illinois Teste de Habilidades Psicolingüísticas (ITPA))	It assesses linguistic, memory and perceptual abilities of children between 2 and 10 years of age	Bogossian & Santos, 1977	Construct validity: not completely confirmed; Discriminant validity:	More severe forms of FASD might not understand the instruction of the instrument, impeding the test completion. One participant	Garcia et al., 2007

			satisfactory (Bagossian 1977)	realized the test and scored below the normal range in all subtests, except for manual expression. Significant Impairment of abilities involving audition.	
Wisconsin Card Sorting Test (WCST)	Assesses executive functions, cognitive flexibility and reasoning. The battery consists of 64 cards and 4 stimulation cards. The Brazilian standardization assessed individuals between 6 years6month and 17 years and 11 months of age.	Cunha et al., 2005	Test-retest correlation: varies between 0,37-0,72 (Cunha et al., 2005); Discriminant validity: has been confirmed (Cunha et al., 2005)	Cognitive functions below average (Stromland et al., 2015). No statistically significant differences between groups, but slightly lower verbal IQ in the PAE group (Furtado &Roriz, 2016).	Stromland et al., 2015 Furtado & Roriz, 2016
Rey Complex Figure Test	Neuropsychological assessment of visual perception and non-verbal immediate memory. A complex figure is shown to the participant who shall copy the figure and draw it later (memory). Figure A assesses individuals between ages 5-88, Figure B assesses children between 4-8 years of age.	Oliveira et al., 2004	Test-retest reliability: Pearsons coefficient: 0.76 (Oliveira et al., 2004); Internal consistency: Cronbach's alpha: 0.86 (copy) and 0.81 (memory) (Oliveira et al., 2004)	PAE groups had significantly lower performance on copying the figure (constructive praxis), and memory recovery after 3 min (immediate visual recall) compared to Non-PAE group. No differences were found in planning task between groups.(Furtado & Roriz, 2016). Performance was below average. The study does not describe details about the participants' performance (Stromland et al., 2015).	Furtado &Roriz, 2016 Stromland et al., 2015
Raven Colored Progressive Matrices (Special Scale) (Angelini et al., 1999)	Assesses cognitive abilities of children between ages 5-11. It consists of three series of 12 items with increasing difficulty	Angelini et al., 1999.(São Paulo); Bandeira et al., 2004 (Porto Alegre)	Measures four factors and a general factor. The items are correlated (r12 = 0,41; r13 = 0,12; r14 = -0,50; r23 = 0,31; r24 = 0,02; r34 = 0,09), Cronbach's alfa above 0.65 (Pasquali et al., 2002)	Below average. The study does not describe details about the participants' performance (Stromland et al., 2015). Lower IQ scores of institutionalized group with European ancestry (Momino et al., 2012)	Stromland et al., 2015 Momino et al., 2012

d2 Test of Attention	Consist of the fast-paced presentation of 14 trials with 60 symbols The participant shall detect the target letter "d" while distracting configurations are presented. The test assesses scanning accuracy, speed, learning, and test-taking strategies. assesses attentional characteristics of individuals between 7 and 76 years old.	Malloy-Diniz et al., 2019	Internal consistency: Cronbach's α = 0.43–0.96; Split-half = 0.40–0.93	PAE groups had higher speed performance with higher error rates as well. The group differences were statistically significant.	Furtado &Roriz, 2016
Rey Auditory Verbal Learning Test (RAVLT)	Tests episodic memory of individuals between 16 and 93 years of age. It uses list-learning paradigm: On list A, 15 words are presented to the subject and than asked to recall the words. The lust B (interference) contains 15 words as well and the participant is asked to recall as many words as possible from the list. After that, the participant is asked to recall immediatley words from the list A.	Malloy-Diniz et al., 2000	High internal consistency: Cronbach's alpha of 0.831 (Paula et al., 2012); Construct validity: Positive correlations between A7-RAVLT and DR-BCSB ($r = .528$, $p < .01$) and between REC A-RAVLT and REC-BCSB ($r = .197$, $p < .01$) (Fichman et al., 2010)	No signiticant differences have been found between groups	Furtado & Roriz, 2016
Test of Semantic and phonetic Verbal Fluency (animals)	The test is part of the Boston Diagnostic Aphasia Examination and the Protocol of Consortium to Establish a Registry for Alzheimer's Disease (CREAD). The standardization study of the test on the Brazilian population was obtained with the participation of individuals from zero to 16 years old. The test consists of Semantic and Phonetic categorization. In the Semantic categorization, the participant is asked to name words in one	Brucki et al., 1997; Malloy-Diniz et al., 2007	Strong internal validity (Oliveira et al., 2016; Passos et al., 2011)	Higher semantic fluency in the non-PAE group. No difference was found in phonemical fluency between groups.	Furtado & Roriz, 2016

	semantic category (animals) within a determined temporal frame. In the Phonetic tests, the participant is asked to name orally words beginning with the letters F, A, or S (the most frequently used words in Portuguese).				

Abbreviations: PAE – Prenatal Alcohol Exposure; FASD- Fetal Alcohol Spectrum Disorder; FAS-Fetal Alcohol Syndrome.

IV. General discussion and future directions

The general objective of the present dissertation was to explore the effects of prenatal alcohol exposure on the developing CNS. In the first article, the aim was to demonstrate novel findings of the combined alcohol and cannabis exposure in utero. The article discusses the interaction of alcohol and marijuana that synergistically alters normal development through the endocannabinoid system, resulting in craniofacial dysmorphism and neurobehavioral changes in the offspring. The figures illustrate the teratogenic mechanisms, as well as the craniofacial alterations caused by the co-exposure of the two substances. The outcomes may vary depending on the quantity, timing of exposure, and constituents of the cannabis products. For this reason, the study summarizes the different exposure procedures from the existing preclinical studies on combined exposure and relates the corresponding outcomes in Table A.

The second article sought to summarize the existing literature on prenatal alcohol exposure in Brazil and to gather information about the neuropsychological assessment tools used in these studies. As it is mentioned in the second article, Brazil has continental dimensions, with a large socioeconomic variety (Guerra et al., 2022). Low socioeconomic status is associated with more severe forms of FASD (May et al., 2022; May et al., 2011; Astley et al., 2000; Thanh et al., 2013). Misuse of substances during pregnancy and its effects on the fetus are understudied topics in Brazil, however, the recent covid-pandemic possibly had an impact on alcohol consumption and unwilled pregnancies in Brazil - as it was observed worldwide (Sher, 2020). The increase in substance misuse (World Drug Report 2022; Bastos, 2017), especially during pregnancy in Brazil (Rodrigues et al., 2019; Silva et al., 2021) warrants the development of new studies on the effects on the offspring, considering the cultural and socioeconomic particularities of the country.

It is suggested that the two substances may disrupt normal myelination - through activation of CB1 receptors - and lead to abnormal white matter formation (Wade et al., 2020). During the brain growth spurt (equivalent to the third trimester of human gestation which continues during adolescence), alcohol alters activity-dependent neuronal plasticity – a crucial process to establish precise synaptic networks between distant brain areas (Tang et al., 2018; Huberman et al., 2008; Kirby et al., 2013).

Preclinical studies shed light on the mechanisms by which ethanol alters the sensory processing of exposed fetus (Medina & Krahe, 2008; Wong et al., 2018; Delatour et al., 2019; Delatour et al., 2020;). Sensory anomalies have been described previously in the FASD literature (Jirikowich et al., 2008; Tesche et al., 2014; Coffman et al., 2020), but novel data enable us to understand the specific mechanisms underlying atypical white matter formation (Tang et al., 2018; Keum et al., 2023). Developmental alcohol exposure in ferret resulted in increased functional connectivity between the caudal and rostral portions of the posterior parietal cortex - areas that play a role on multisensory integration (MSI) (Tang et al., 2018). More recently, Keum and colleagues (2023) demonstrated that developmental alcohol exposure disrupts MSI in the ferret. Alteration in white matter integrity is associated with lower cognitive functioning, which is reflected in lower scores on cognitive assessments and higher rates of maladaptive behavior (Fan et al., 2016; Manouilenko et al., 2013; Jirikowic et al., 2008).

Thus, altered white matter integrity should be taken into consideration during neuropsychological evaluation and/or special educational care for individuals with developmental history of alcohol and cannabis exposure. Therefore, future studies are encouraged to consider the multisensory processing characteristics of FASD individuals, as well as the construction and validation of new assessment tools to investigate multisensory processing particularities in children with prenatal exposure.

FASD is an irreversible condition that has lifelong impact on the individual's life and on the society. Even though no consensus exist on ideal therapeutic approaches, some pharmacological (for a comprehensive review see: Ritfield et al., 2022) and other alternative interventions may improve the deficits seen in FASD individuals such as environmental enrichment (Wang et al., 2022; Kelly et al., 2009; Gursky & Klintsova, 2017), aerobic exercise (Gursky & Klintsova, 2017; Milbocker et al., 2022), cognitive-behavioral methods (Kulberg & Buckley 2007; Peadon et al., 2009), and self-regulation intervention programs (Kable et al., 2015; Reid et al., 2017). Therefore, interventions addressing specific functional disabilities of FASD are promising and their implementation in the Brazilian public health practice is needed.

V. References

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